

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF
SCIENCE ENGINEERING AND TECHNOLOGY

INVESTIGATING THE PHENOLIC CONTENT AND *IN VITRO*
BIOACCESSIBILITY OF SOME VINEGARS, AND CHANGES IN ANTIOXIDANT
ACTIVITY DURING GRAPE AND APPLE VINEGAR PROCESSING

M.Sc. THESIS

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Department of Food Engineering

Food Engineering Programme

JANUARY 2014

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**BAZI SİRKE ÇEŞİTLERİNİN FENOLİK MADDE İÇERİĞİ VE *İN VİTRO*
BİYOERİŞEBİLİRLİĞİNİN VE ÜZÜM İLE ELMA SİRKESİ ÜRETİMİ SIRASINDA
ANTİOKSİDAN AKTİVİTEDE MEYDANA GELEN DEĞİŞİMLERİN İNCELENMESİ**

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Sena BAKIR, a **M.Sc.** student of ITU Graduate School of Food Engineering student ID 506121525, successfully defended the **thesis** entitled “**Investigating the Phenolic Content and *In Vitro* Bioaccessibility of Some Vinegars, and Changes in Antioxidant Activity During Grape and Apple Vinegar Processing**”, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my beloved family,

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ABBREVIATIONS

ABTS: 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid

ANOVA : Analysis of Variance

CA : Cateshin Equavelent

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EtOH: Ethhyl Alcohol

GAE: Gallic Acid Equivalent

GI: Gastrointestinal

HPLC: High Performance Liquid Chromotography

IN: Solution Entered The Dialysis Tubing

MeOH: Methyl Alcohol

MQ: Milli-Q water

OUT: Solution Remained in The Dialysis Tubing

PG: Post Gastric

SD: Standard Deviation

SPSS: Statistical Package for the Social Sciences

TEAC: Trolox Equivalent Antioxidant Activity

TFC: Total Flavonoid Content

TPC: Total Phenolic Content

TPTZ: 2,4,6-Tripyridyl-s-Triazine

Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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SUMMARY

The vinegar as old as wine history, has a lot of positive effect on health is defined by researchers. It is considerable that antioxidants occurred in vinegar have a good effect on health.

There is limited information in the literature on comparison of antioxidant properties of wine and vinegar. However, information on the influence of processing steps of industrial vinegar production on phenolic compound and antioxidant content is missing. In addition to this there is not any document about how much of antioxidant material could be digested in the case of consumption of vinegar.

The aim of this study is to provide data on total flavonoid and total phenolic content and also antioxidant capacity of raw materials used for vinegar production, ready to consume last products of vinegars, and to practice *in vitro* bioaccessibility analyses to some selected vinegars. Additionally, phenolic material content of 18 different vinegar samples collected from different companies were compared with each other.

While investigating the process effect on phenolic material content, grape and apple vinegars were preferred. Fruit concentrate, fruit wine, not decanted-not filtered fruit vinegar, decanted-not filtered fruit vinegar, filtered fruit vinegar and ready to consume fruit vinegar were used as intermediate product steps of consecutive products.

In the case of comparison of grape wine and grape vinegar on dry weight basis, it was observed that there is a decrease of 8.3% for phenolic content and increase of 5.8% for flavonoid content. Results of the study indicated there is an exact decline of ABTS values from 112.11 ± 24.62 mg TEAC/100 mL fresh sample to 43.89 ± 7.06 mg TEAC/100 mL fresh sample, CUPRAC values from 118.29 ± 13.63 mg TEAC/100 mL fresh sample to 76.11 ± 10.33 mg TEAC/100 mL fresh sample, DPPH values from 105.72 ± 1.88 mg TEAC/100 mL fresh sample to 60.84 ± 3.88 mg TEAC/100 mL fresh sample and FRAP values from 32.45 ± 1.79 mg TEAC/100 mL fresh sample to 21.56 ± 2.41 mg TEAC/100 mL fresh sample ($p < 0.05$). Taking into consideration of dry content based results, these values have decreased. Similar losses were observed in apple vinegar processing steps; however there was some increase in antioxidant capacity on dry weight basis.

In vitro bioaccessibility assay was performed on apple vinegar, grape vinegar, pomegranate vinegar and balsamic vinegar. It was observed that, in the event of investigation of flavonoid and phenolic content, and antioxidant capacity recovery rate, apple vinegar generally has highest values among all analysis. However, total phenolic content and FRAP assay results of the grape vinegar were higher than those of apple vinegar (83.6% and 12.5%, respectively). On the other hand, balsamic vinegar had the maximum recovery rate with values of 3.05 ± 0.44 mg CA/100 mL fresh sample for flavonoid test, 34.12 ± 2.87 mg GAE/100 mL fresh sample for phenolic test, 13.07 ± 0.54 mg TEAC/100 mL fresh sample for ABTS test, 48.19 ± 9.60 mg TEAC/100 mL fresh sample for CUPRAC test, 6.46 ± 2.79 mg TEAC/100 mL fresh sample for DPPH test, and 11.24 ± 1.58 mg TEAC/100 mL fresh sample for FRAP test ($p < 0.05$).

Apple vinegar, grape vinegar, pomegranate vinegar, balsamic vinegar produced with industrial fast manufacturing type and grape vinegar, apple vinegar, gilaburu vinegar, pomegranate vinegar, artichoke vinegar, rosehip vinegar, blueberry vinegar, lemon vinegar, blackberry vinegar, mulberry vinegar, rice vinegar, apricot vinegar, date vinegar and hawthorn vinegar produced with old fashion fermentation in woods were utilized for comparison of different vinegar samples.

In conclusion, balsamic vinegar had the highest flavonoid and phenolic contents, sequentially 96.13 ± 18.31 mg CA/100 fresh sample and 254.66 ± 24.38 mg GAE/100 mL fresh sample. In the case of crosscheck of antioxidant capacity, balsamic vinegar had the highest values in CUPRAC and FRAP assays, 708.67 ± 107.83 and 420.84 ± 28.37 mg TEAC/100 mL, respectively, on the other hand blueberry vinegar had the highest value with 373.32 ± 19.01 mg TEAC/100 mL fresh sample for ABTS analysis and finally rosehip vinegar had the highest value with 517.05 ± 43.40 mg TEAC/100 mL fresh sample for DPPH analysis ($p < 0.05$).

Phenolic profiles of all samples were also evaluated by HPLC-PDA for vinegar processing, bioaccessibility of vinegars -with initial values and, PG, IN and OUT fractions- and different types of vinegars. As a consequence of most vinegar substitutes contain similar phenolic compounds such as gallic acid, protocatechuic acid, p-hydroxybenzoic acid, (+)-catechin, syringic acid, caffeic acid, p-coumaric acid, specific phenolics were determined in this study.

**BAZI SİRKE ÇEŞİTLERİNİN FENOLİK MADDE İÇERİĞİ VE *IN VITRO*
BİYOERİŞEBİLİRLİĞİNİN VE ÜZÜM İLE ELMA SİRKESİ ÜRETİMİ SIRASINDA
ANTIOKSİDAN AKTİVİTEDE MEYDANA GELEN DEĞİŞİMLERİN İNCELENMESİ**

ÖZET

Tarihi şarap kadar eski olan sirkenin sağlık üzerine pek çok olumlu etkisinin olduğu araştırmacılar tarafından belirtilmektedir. Sirkelerde bulunan antioksidanların da sirkenin sağlık üzerindeki olumlu etkisine katkı yaptığı düşünülmektedir.

Sirkelerde bulunan antioksidan özelliklerin şaraplarla kıyaslanması hakkında literatürde az da olsa çalışma bulunabiliyorken, endüstriyel ölçekte sirke üretimi sırasında, proses basamaklarının ilk üründen son ürüne kadar fenolik madde ve antioksidan içeriğine ne gibi etkileri olduğu ile alakalı bir çalışma bulunamamıştır. Buna ilaveten sirkenin tüketilmesi durumunda sirkede başlangıçta var olan antioksidan maddelerin ne kadarının sindirilebileceği ile alakalı olarak da literatürde herhangi bir çalışmaya rastlanamamıştır.

Bu çalışma ile sirke üretiminde kullanılan hammaddelere, üretim sırasında elde edilen ara ürünlere, piyasaya sürülme aşamasındaki son ürünlere ve seçilen bazı sirke numunelerine *in vitro* biyoerişebilirlik analizi uygulanarak toplam flavoid, toplam fenolik madde içerikleri ile antioksidan kapasiteleri konusunda bilgi edinilmesi amaçlanmıştır. Tüm bunlara ilaveten piyasadan toplanan 18 farklı sirke numunesi de fenolik madde içerikleri bakımından birbirleri ile kıyaslanmıştır.

Proses basamaklarının fenolik madde içeriğine etkisi incelenirken üzüm ve elma sirkesi tercih edilmiş, ara basamak olarak da meyve konsantresi, meyve şarabı, durultulmamış-filtre edilmemiş meyve sirkesi, durultulmuş-filtre edilmemiş meyve sirkesi, filtre edilmiş meyve sirkesi ve tüketime hazır meyve sirkesi kullanılmıştır.

Yapılan analizlerde üzüm şarabı ile üzüm sirkesi aşamaları kuru maddede bazında kıyaslandığında toplam fenolik madde içeriğinin %8.3 düştüğü, toplam flavonoid madde içeriğinin ise %5.8 oranında yükseldiği gözlemlenmiştir. Antioksidan kapasite değerleri incelendiğinde ise ABTS değerlerinin 112.11 ± 24.62 mg TEAC/100 mL taze örnek değerinden 43.89 ± 7.06 mg TEAC/100 mL taze örnek değerine, CUPRAC değerlerinin 118.29 ± 13.63 mg TEAC/100 mL taze örnek değerinden 76.11 ± 10.33 mg TEAC/100 mL taze örnek değerine, DPPH değerlerinin 105.72 ± 1.88 mg TEAC/100 mL taze örnek değerinden 60.84 ± 3.88 mg TEAC/100 mL taze örnek değerine ve FRAP değerlerinin de 32.45 ± 1.79 mg TEAC/100 mL taze örnek değerinden 21.56 ± 2.41 mg TEAC/100 mL taze örnek değerine düştüğü görülmüştür ($p < 0.05$). Kuru madde içeriği göz önüne alındığında ise bu değerlerde artış değil azalma gözlemlendiği de belirtilmelidir. Elma sirkesi üretimine proses etkisi incelendiğinde de benzer şekilde basamaklar arası değer

kayıpları gözlemlenmiş fakat kuru madde içeriği göz önünde bulundurulduğunda antioksidan kapasitede artış elde edilebilmiştir.

In vitro biyoerişebilirlik analizi elma sirkesi, üzüm sirkesi, nar sirkesi ve balsamik sirkeye uygulanmış olup flavonoid fenolik ve antioksidan kapasitesinin geri kazanım oranları incelendiğinde ise elma sirkesinin tüm analizlerde en yüksek orana sahip olduğu ancak üzüm sirkesinin toplam fenolik analizinde %83.6, FRAP analizinde ise %12.5 ile elma sirkesini geçtiği görülmüştür. Buna rağmen miktarlar incelendiğinde ise balsamik sirkenin toplam flavonoid içeriği analizinde 3.05 ± 0.44 mg CA/100 mL taze örnek, toplam fenolik içeriği analizinde 34.12 ± 2.87 mg GAE/100 mL taze örnek, ABTS analizinde 13.07 ± 0.54 mg TEAC/100 mL taze örnek, CUPRAC analizinde 48.19 ± 9.60 mg TEAC/100 mL taze örnek, DPPH analizinde 6.46 ± 2.79 mg TEAC/100 mL taze örnek, FRAP analizinde ise 11.24 ± 1.58 mg TEAC/100 mL taze örnek değerleri ile en yüksek geri kazanıma sahip olduğu söylenebilmektedir ($p < 0.05$).

Farklı sirke örneklerinin kıyaslanmasında ise endüstriyel hızlı tipte üretilen elma sirkesi, üzüm sirkesi, nar sirkesi ve balsamik sirke ile, eski tipte doğal fermentasyon yoluyla fıçılarda üretilen üzüm sirkesi, elma sirkesi, gılaburu sirkesi, nar sirkesi, enginar sirkesi, kuşburnu sirkesi, yaban mersini sirkesi, limon sirkesi, böğürtlen sirkesi, dut sirkesi, pirinç sirkesi, kayısı sirkesi, hurma sirkesi ve alıç sirkesi kullanılmıştır.

Yapılan kıyaslama neticesinde balsamik sirkenin 96.13 ± 18.31 mg CA/100 mL taze örnek ile 254.66 ± 24.38 87 mg GAE/100 mL taze örnek değerleri ile sırayla en yüksek flavonoid ve fenolik madde içeriğine sahip olduğu görülmüştür. Antioksidan kapasitelerin kıyaslanmasında kullanılan analizler incelendiğinde ise balsamik sirkenin CUPRAC ve FRAP analizlerinde 708.67 ± 107.83 ve 420.84 ± 28.37 mg TEAC/100 mL değerleriyle sirkeler arasında en yüksek değere sahip olduğu görülürken, ABTS analizinde 373.32 ± 19.01 mg TEAC/100 mL taze örnek değeri ile yaban mersini sirkesinin, DPPH analizinde ise 517.05 ± 43.40 mg TEAC/100 mL taze örnek değeri ile kuşburnu sirkesinin en üst değerlere sahip bulunduğu belirtilmelidir ($p < 0.05$).

Tüm bunlara ilaveten sirke üretim prosesinin alt basamakları, *in vitro* biyoerişebilirlik analizinin tüm fraksiyonları ile karşılaştırmada kullanılan tüm sirkelere HPLC-PDA analizi uygulanarak tüm numunelerin fenolik profillerinin belirlenmesi de amaçlanmıştır. bu amaçla sirkelerde genelde ortak olarak bulunan fenolikler belirlenmiş ve belirlenen fenoliklerin numunelerde bulunup bulunmadığı araştırılmıştır.

Elde edilen sonuçlar ışığında üzüm şarabının gallik asit içeriği çok yüksek bulunmakla beraber, asetik asit fermentasyonunu takiben gallik asit içeriğinin hızla düştüğü gözlemlenmiştir. Elma sirkesi proses basamaklarında ise üzüm sirkesi proses basamaklarından farklı fenoliklere de rastlanmıştır. Elma suyu konsantresinde gallik asit, *p*-hidroksibenzoik asit, kateşin, siringik asit, kafeik asit, ve *p*-kumarik aside rastlanmıştır.

In vitro biyoerişebilirlik analizlerinin fraksiyonların kromatogramları incelendiğinde balsamik sirke de gallik asit, kafeik asit ve *p*-kumarik aside, nar sirkesinde allik asit, protokateşuik asit, *p*-hidroksibenzoik asit, kateşin, siringik aside rastlanmıştır.

Biyoerişebilirlik analizine alınan tüm sirkelerin HPLC-PDA verileri incelendiğinde ise başlangıç değerlerinin *in vitro* biyoerişebilirlik analizinden sonra genel olarak kaybolduğu görülmüştür.

Analize dahil edilen 18 sirkenin fenolik profillerinin birbiri ile karşılaştırılması sonucunda balsamik sirkenin en yüksek gallik asit seviyesine sahip olduğu buna rağmen protokateşuik asit, *p*-hidroksibenzoik asit, kateşin konsantrasyonlarına sahip olmadı gözlemlenmiştir. Yabanmersini sirkesinin 60.08 ± 12.93 mg gallik asit/100 mL konsantrasyonu ile balsamik sirkeyi takip ettiği, ayrıca 18.74 ± 0.12 mg/100 mL değeri ile en yüksek protokateşuik asit değerine sahip olduğu görülmüştür.

Gilaburu sirkesi ve nar sirkesi I diğer sirkelerden daha fazla *p*-hidroksibenzoik asit değerine sahipken, nar sirkesi I ve üzüm sirkesi II sahip oldukları kateşin konsantrasyonu ile diğer sirkeleri geride bırakmaktadır.

1. INTRODUCTION

Vinegar was known since old Oriental civilizations and was employed as a poor man's drink and later as a remedy in ancient Greece and Rome. Vinegar is the most important single flavoring used to provide or enhance the sour, acidic taste of food (Belitz et al., 2004). Nowadays vinegar has an important role in gastronomy and its quality is protected by title of origin in different vinegar-producing areas (Tesfaye et al., 2009).

Vinegar is commonly used as an ingredient in the food systems (Xu et al., 2007), and it is a good solvent for the essential oils of herbs and spices and has been a ubiquitous sauce ingredient throughout history (Adams, 1985). Besides this vinegar has some health effect; such as promoting recovery from exhaustion (Fushimi et al., 2001), regulating blood glucose (Ebihara & Nakajima, 1988), blood pressure (Kondo et al., 2001), aiding digestion (Liljeberg and Bjorck, 1998), stimulating the appetite, and promoting calcium absorption (Kishi et al., 1999).

Acetic acid is the major volatile acid in wine and it is also an active ingredient in household vinegar, and common white household vinegar consists of approximately 5% acetic acid. Friedman et al., (2006) claims that acidity, alcohol content, and content of polyphenolic flavonoid compounds including tannins and resveratrol may be responsible for antimicrobial activities reported for wine against foodborne pathogens. Because of this idea these wine substances, vinegar, would then be regarded as nontoxic, food-compatible, and plant-derived antimicrobials (Theron and Rykers-Lues, 2010).

Cerezo et al., (2008) studied some red wine vinegars and indicated some phenolic compounds in different finished vinegars as gallic acid, protocatechuic acid, tyrosol, caftaric acid, vanilic acid, (+)- catechin, caffeic acid, syringic acid, gallic ethyl ester,

(-)-epicatechin, resveratrol glucoside, ellagic acid. Because of its beneficial health effects, vinegar found a wide application area such as pickles, salads, souces, and medicine. However there is not adequate source to illitarete antioxidant properties of vinegar.

The aim of this study is to firstly determine the process effect on total flavonoid, total phenolic content and antioxidant capacity of the selected vinegars. For this purpose, different steps of production process of vinegar were analyzed with several methods. In addition to the process effect, as there is a lack of *in vitro* bioaccessibility of vinegar in literature, second target of this research is evaluating the *in vitro* bioaccessibility of some chosen vinegars with the aspect of phenolic content. Finally, this work focused on the comparasion of different vinegars' antioxidant content, which were collected from varied companies.

2. LITERATURE REVIEW

2.1 History of the Vinegar

Ethnic fermented foods have been prepared and consumed for centuries for nutritional properties, stability, taste, aroma, and flavor, and also for therapeutic purposes. Fermented foods are biologically important because of some of these properties. Vinegar has been used as a condiment, a preservative, and a medicine since ancient times (Tamang and Kailasapathy, 2010).

Origin of the vinegar is extend to Vin aigre in French means ‘the sour wine’ (Ozturk et al., 2009). Vinegar is generally described as a liquid which contains at least four percent acetic acid and sometimes also other aroma and flavour compounds, but no nutritious substances (Horwood, 1990). According to the TS 1880 EN 13188, vinegar is defined as a product is gained from must, the resulting sequence of applying various pre-treatment to grapes or the fruit or sweet or starchy materials with sugar, with firstly ethyl alcohol fermentation and then acetic acid fermentation.

Besides grape and apple, banana, lemon, strawberry, rice, orange, howthorne, pineapple, mulberry, blueberry, blackberry or some other fruits and vegetables can be utilized as a raw material in vinegar production all around the World (Maldonado vd., 1975; Nakayama, 1980, Shaw, 1983). Vinegars are named according to their raw material and they have properties of the corresponding raw material (Elgun, 2011 and Catsberg et al., 1990).

The top 20 contries for 2011 and 2012 values of vinegar and substitutes for vinegar from acetic acid, export volume (liters) are shownin Figure 2.1. Recorded data

indicated that Italy had the biggest amount of export volume with approximately 112 million liters on vinegar and substitutes for vinegar from acetic acid. This country was followed by Spain and Greece with 43 million and 41 million liters, respectively. Turkey ranked at 19th with 2.6 million liters (Factfish, 2011a).

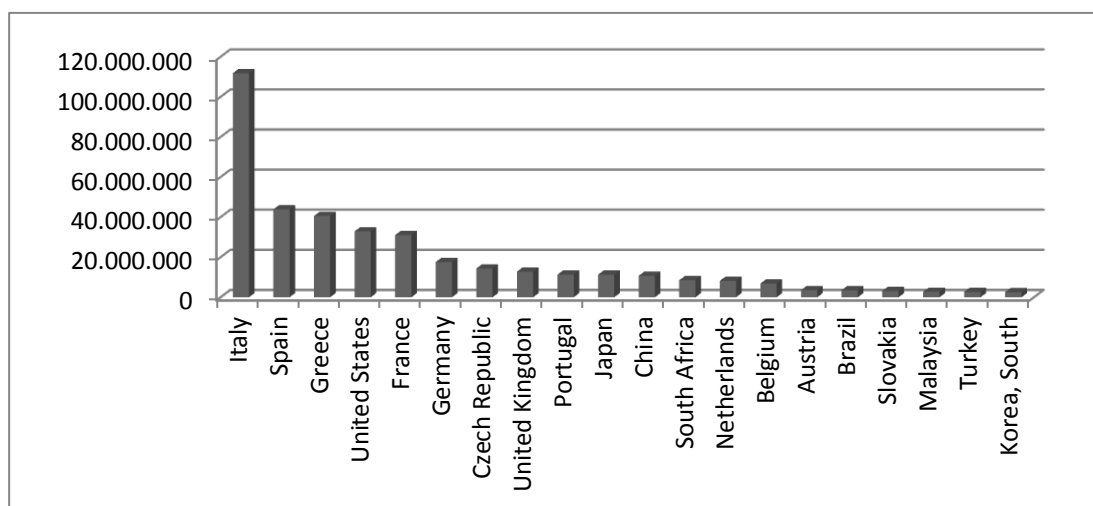


Figure 2.1 : The top 20 countries for 2011 and 2012 values of vinegar and substitutes for vinegar from acetic acid, export volume (liters) (Factfish, 2011a).

The top 20 countries for 2011 and 2012 latest values of vinegar and substitutes for vinegar from acetic acid, import volume (liters) are shown in Figure 2.2. The United States had the highest amount of import value with approximately 67 million liters on vinegar and substitutes for vinegar from acetic acid. The United States was followed by Germany, France and Italy. The import values of vinegar in Turkey recorded as ≈ 250 thousand liters, so Turkey replaced at 89th at the order (Factfish, 2011b).

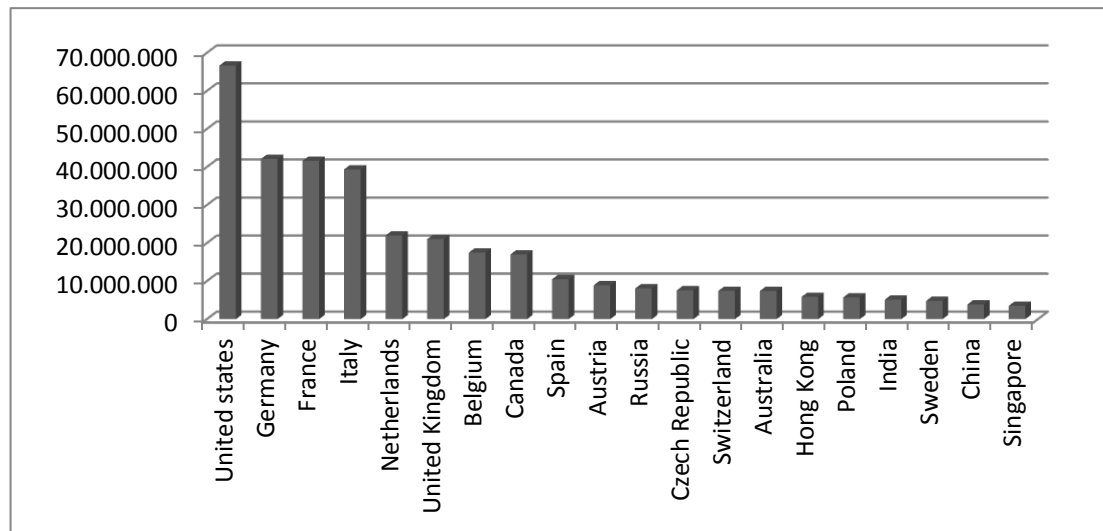


Figure 2.2 : The top 20 contries for 2011 and 2012 values of vinegar and substitutes for vinegar from acetic acid, import volume (liters) (Factfish, 2011b).

2.2 Production of the Vinegars

2.2.1 Production of Vinegar

Vinegar is a nonalcoholic product, but its production steps involve an alcoholic fermentation stage (Lee, 1983). As mentioned before, vinegar can be produced from fermented apples, wine, or other raw materials after alcoholic fermentation. Numerous species of the genus *Saccharomyces* such as *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus*, ferment sugar and yield ethyl alcohol and carbon dioxide (Potter et al., 1998). The acetic acid fermentantion is made by numerous species of the genus *Acetobacter*, for example, *A. aceti*. The acetic acid bacteria convert alcohol into acetic acid to obtain the energy necessary to carry on their life processes by oxidation (Lee, 1983).

2.2.2 Production Methods of Vinegar

Vinegar can be produced by several methods such as traditional slow method, Pasteur method, Orleans method, fast (generator) method and submerge method. The wine that is going to be processed into vinegar must contain 7-7.5% alcohol. Therefore, initial alcohol content of wine which is about 11-12%, is reduced before vinegar processing (Tosun, 2011 and Elgun, 2011).

2.2.2.1 Traditional Slow Method

Alcohol fermentation is carried out until alcohol concentration reaches around 13% level at first step. Following alcohol fermentation acetic acid bacteria grow on the surface of the liquid. These bacteria use ethyl alcohol and turn it into acetic acid. This method allows slow production of vinegar however quality of vinegar is highly rated and can be used to produce aromatic vinegars. Wooden barrels or tanks can be used in this method. Generally, these tanks have 200-300 L volume. The air holes, 2-3 cm diameter, are opened at 3-5 cm above of wine surface or 2/3 of barrels and a funnel is placed at the hole located at top. Handle of the funnel reaches the inside of the wine. A board taps is integrated to the barrel. Half of barrel is filled with wine and in proportion of $\frac{1}{3}$ - $\frac{1}{4}$ non-pasteurized raw vinegar is added over wine, leaves for vinegar processing occurs at 28-30°C for 6-8 weeks. The membrane, generated by acetic acid bacteria, precipitates because of density difference of acetic acid and alcohol. This precipitation indicated the end of vinegar processing (Tosun, 2011 and Elgun, 2011).

2.2.2.2 Pasteur Method

This method is based on connecting the barrels at traditional slow method together with pipes and actualizing a continuous system. Processing of vinegar occurs in a circle (Tosun, 2011 and Elgun, 2011).

2.2.2.3 Orleans Method

Barrels of 220-230 L volume are utilized for this method and are placed in a row horizontally. Barrels used for vinegar production in Orleans method are shown in Figure 2.3. Front side of barrels have two holes about 6-7 cm diameter. One of these holes is called eye and is used for filling wine and discharging vinegar, the other hole is called stopper and is used for air intake. 150 L of vinegar which is at vinegar degree 8 contains lots of acetic acid bacteria, prepared for vinegar processing, and 10 liters of wine is added to it after every 8 days. This procedure is applied until the liquid level reaches the 5 cm below the eye. Vinegar processing is accomplished after 15 days, and 10 liters of vinegar is discharged from barrel and replaced with 10 liters of wine (Tosun, 2011 and Elgun, 2011).



Figure 2.3 : Barrels used for vinegar production in Orleans method (Tosun, 2011).

2.2.2.4 Fast (Generator) Method

Fast method is commonly used in Turkey. The system used for vinegar production in fast (generated) method is shown in Figure 2.4. The filling materials; e.g. chip, corncop without grains, polyurethane foam, allow immobilizing the bacteria in the fermentor cabin and supply large area for bacteria. The wine is slowly drained at the surface of the immobilized bacteria. The air requirement is supplied from the holes placed at cider of the fermentor cabin. The vinegar produced is picked up from the bottom of the tank. Diameter of this generator change among 0.8-3 m and height of it could be 2-12 m. The filling materials is treated with raw vinegar or air holes of the tank closed and tank filled with raw vinegar for fastening the bacterias these materials before exposing with wine. The next step is streaming the wine intermittently over the bacteria for 7-10 days. As the rain streamed wine is turned into vinegar by immobilized cells. The generetor method enables oxidating 2.5-3 L pure alcohol with 1m³ filling material per day, and also this method can be sustained with a motor which aggregates the wine, not turned into to vinegar. This system is called as “Fring Generator” and rantability of the vinegar increses and shortens production time (De Ory et al., 2004, Tosun, 2011 and Elgun, 2011).

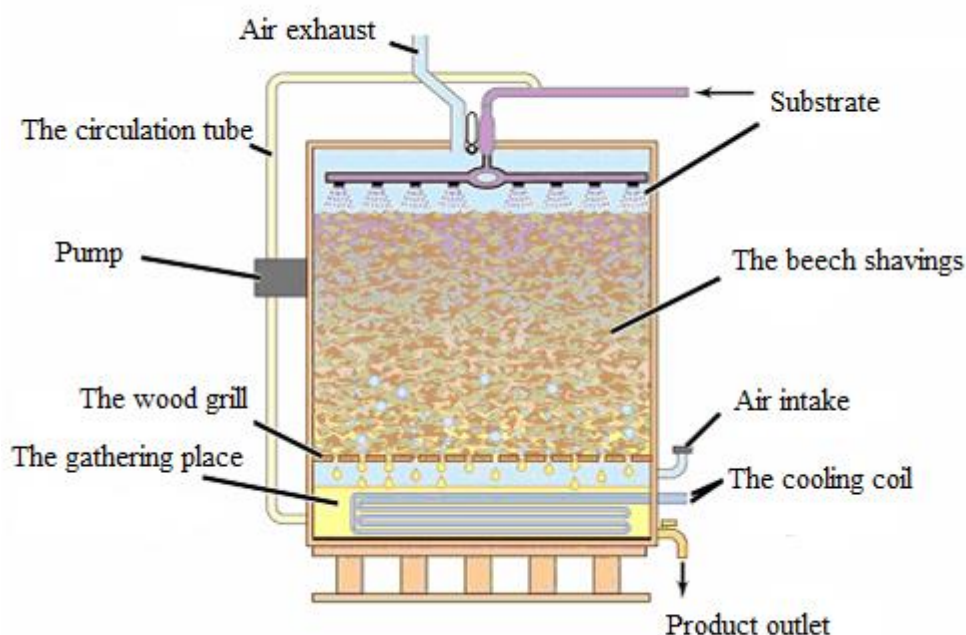


Figure 2.4 : The system used for vinegar production in fast (generated) method (Tosun, 2011).

2.2.2.5 Submerge Method

This method facilitates multiplying bacteria in the substrate without filling materials. The speed of this method is 30 times more than generator method. Fermentation is actualized between 24-29°C with 8-12% of wine continuously stirred. Vinegar processing occurs in the wine, not at the surface of substrate. The required oxygen is given to the environment in a controlled manner. The fermentation tank should be made of stainless steel, wooden or synthetic material resistant to acidic environment. The tank contains pH, temperature, alcohol and air amount indicator systems. Using this method, 5-10 tones of vinegar containing 4-5% acetic acid can be produced in 24 hours. After production vinegar is left for settling. Remained alcohol react with acids and forms esters. Clarification process of the vinegars is made by microfiltration and this step eliminates the pasteurization or sterilization of vinegars. The acidity, ash content and alcohol content of final product is around 4%, 0.8 g/L and 0.1%, respectively (Tosun, 2011 and Elgun, 2011).

2.2.3 Production of Balsamic Vinegar

Unlike other vinegars, traditional balsamic vinegar production can be divided into four main steps; cooking of grape juice, cooked must fermentation, acetic oxidation, and slow aging of vinegar. The cooking of the grape juice takes between 12 and 24 h and produces chemical and physical modifications that strongly affect the successive stages of traditional balsamic vinegar production. After cooking, the must conducts alcoholic fermentation of sugars by yeasts, followed by acetic oxidation of the ethanol by acetic acid bacteria, both biological processes taking place in a cask, the “badessa.” These two biological processes take more or less 1 year to be fully completed. The alcoholic conversion is easier to control than that of the acetic acid, which is a serious problem for traditional balsamic vinegar production because incomplete oxidation of the ethanol produces vinegars with low titratable acidity, affecting negatively the sensory perception of the end quality. Finally, such vinegar undergoes slow aging in the barrel set to concentrate flavors. Aromatic compounds accumulate and intensify over decades, with the vinegar kept in fine wooden casks becoming sweet, brown, viscous, and concentrated. The aging of vinegar is the longest step and it occurs inside a set of barrels of different volumes, made of different types of wood (Verzelloni et al., 2007 and Guidici et al., 2009).

2.3 Chemical and Physical Properties of Vinegar

The Turkish Food Codex Regulation, other than Colours and Sweeteners in Food Additives Notification indicates the allowed maximum amount of sulfur dioxide (SO₂), food preservative as 170 mg/L, and accordance with the Turkish Food Codex Regulation, Contaminants Notification, permitted metal and metalloid maximum quantities of iron is 10 mg / L, copper-zinc is 10 mg / L, for lead 1 mg / L, and for arsenic 1 mg / L to engage in vinegar.

2.4 Health Effects of Vinegar

The utilization of vinegar to fight infections and other acute conditions dates back to Hippocrates (460–377 BC) who recommended a vinegar preparation for cleaning ulcerations and for the treatment of sores. It was observed that vinegar has a good influence on rats while investigating the cardiovascular effect of it. According to a

recent study, spontaneously hypertensive (SHR) rats fed a standard laboratory diet mixed with an acetic acid solution or deionized water, a significant reduction in systolic blood pressure (~20 mmHg) was noted for the SHR rats fed the acetic acid (Johnstone, 2006). In addition to these, vinegar is commonly used as an ingredient in the food systems (Xu et al., 2007), and it is a good solvent for the essential oils of herbs and spices and has been a ubiquitous sauce ingredient throughout history (Adams, 1985). Besides this vinegar has some health effects; such as promoting recovery from exhaustion (Fushimi et al., 2001), regulating blood glucose (Ebihara & Nakajima, 1988), blood pressure (Kondo et al., 2001), aiding digestion (Liljeberg and Bjorck, 1998), stimulating the appetite, and promoting calcium absorption (Kishi et al., 1999) as mentioned at introduction part.

Drinking vinegar has a possitive effect on iron status in non-pregnant women and an intake before or at an earlier stage of pregnancy might prevent iron deficiency in pregnant women (Heins et al., 1999).

2.5 Changes Occurred During Processing

Differences in the antioxidant activities among grape juice, wine, and vinegar were based on to their different phenolic contents and compositions and to other non-phenolic antioxidants present in the samples. Antioxidant activity of grape-derived products is influenced, not only by their content of polyphenols, but also by their phenolic compositions, all of which are influenced by vintage, grape variety, wine and vinegar production method (Da'valos et al., 2005).

During industrial vinegar manufacturing the wine substrate is diluted to reduce the alcoholic degree and promote the growth of acetic bacteria. This practice, therefore, may explain the lower antioxidant activity found in commercial vinegars compared to wines (Cerezo et al., 2010).

Tesfaye et al. (2009), confirmed the presence of natural antioxidants at the end of the production process that transforms grape must into traditional balsamic vinegar from Modena.

Phenolic polyphenols identified in vinegar by several authors are represented below figure 2.5 (Phenol-Explorer, 2014). Amount of these polyphenols show varied values dependent to the vinegar sample. Some authors found very low or none in a vinegar while others found maximum level of it in other vinegar. For example; (+)-Catechin can not be specified in malt vinegar, while Natera et al. (2003) found 8.29 mg/100 mL of (+)-Catechin in apple vinegar by. Gallic acid was not specified in alcohol vinegar (Natera et al., 2003), however Alonso et al. (2004) reported 9.50 mg/100 ml gallic acid in vinegar aged in wood). These findings indicate that each vinegar has different polyphenolic content based on chromatography.

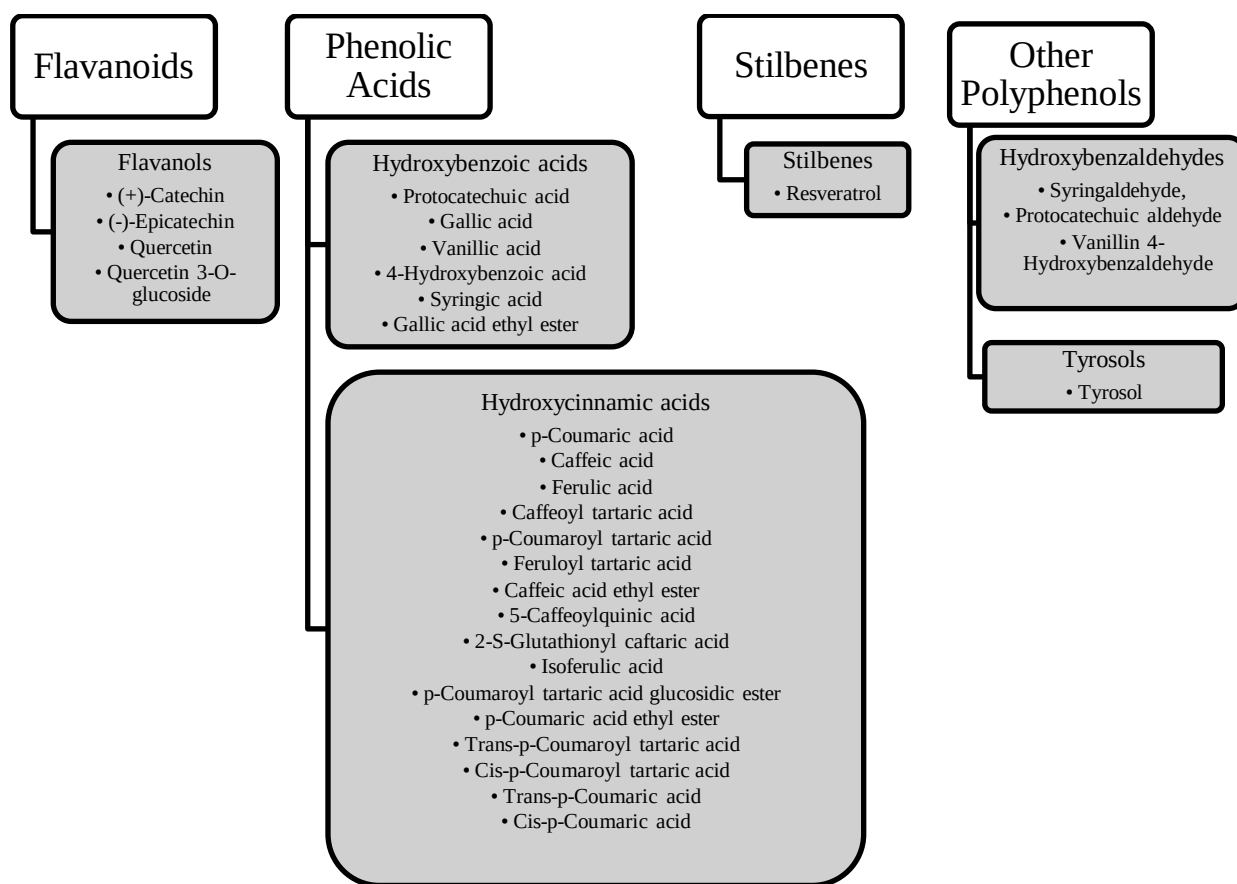


Figure 2.5: Phenolic polyphenols identified in vinegar by several authors (Phenol Explorer, 2014).

3. MATERIALS AND METHODS

3.1 Materials

The vinegar samples used in the analyses are shown in Table 3.1. Products supplied from Kuhne Vinegar Factory, Nahita Natural Fermentation Vinegar Company and Yedier Vinegar Company were provided as at least triple parallel. Experiments were performed in triplicate, mean values were reported.

Table 3.1: Vinegar samples.

Company Name	Product	Ingredients	Batch Number	Date of Production	Date of Last Consumption
Kuhne Vinegar	Apple Vinegar	Apple vinegar, antioxidant (sodium metabisulfite)	050313	05.03.13	05.03.16
	Grape Vinegar	Grape vinegar, antioxidant (sodium metabisulfite)	250213	25.02.13	25.02.16
	Pomegranate Vinegar	Pomegranate vinegar, antioxidant (sodium metabisulfite)	070912	07.09.12	07.09.15
	Aceto Balsamico	Grape vinegar, grape juice concentrate, colorant (caramel), antioxidant (sodium metabisulfite)	250213	25.02.13	25.02.16

Table 3.1(Continued): Vinegar samples.

Company Name	Product	Ingredients	Batch number	Date of Production	Date of Last Consumption
Nahita Natural Fermentation Vinegars	Grape Vinegar	Grape juice	2012-10	10.10.12	Unspecified
	Gilaburu Vinegar	Gilaburu, water	2012-10	10.10.12	Unspecified
	Blackberry Vinegar	Blackberry, water	2012-10	10.10.12	Unspecified
	Artichoke Vinegar	Artichoke vinegar	2013-07	08.07.13	Unspecified
	Lemon Vinegar	Lemon, water	2012-10	10.10.12	Unspecified
	Rosehip Vinegar	Rosehip vinegar	2012-02	10.02.2012	Unspecified
	Howthorne Vinegar	Howthorn berry, water	0911-005	19.09.11	Unspecified
	Apple Vinegar	Apple, water	0911-007	19.09.2011	Unspecified
	Blueberry Vinegar	Blueberry, water	Unspecified	10.13	Unspecified
Yedier Vinegar	Date Vinegar	90% date, 10% water	01.06.2013	01.06.2013	01.06.2015
	Mulberry Vinegar	90% mulberry, 10% water	01.06.2013	01.06.2013	01.06.2015
	Apricot Vinegar	74% apricot, 6% molasses, 20% water	20.09.2012	20.09.2012	20.09.2014
	Rice Vinegar	90% rice, 4%molasses, 6% water	01.06.2013	01.06.2013	01.06.2015
	Pomegranate Vinegar	74% pomegranate,6% molasses, 20% water	20.09.2012	20.09.2012	20.09.2014

3.1.1 Production Process of Kuhne Vinegar Samples

According to information provided by the Kuhne, fruit wine produced by utilizing raw material of vinegar that will be done. In general, apple and grape juices are concentrated with 60-70% efficiency. After this process, fruit concentrate is left for ethyl alcohol fermentation until ethyl alcohol content reaches 8-12% for grape wine and 9-11% for apple wine. Wines obtained with above mentioned method are turned into vinegar with acetic acid fermentation in acetators by dint of yeast. Achieved product is called as "raw vinegar" and this vinegar is taken into resting stage for at least 3 months. Hence, maturation of vinegar is achieved. At the end of 3 months, decantation process is applied to the vinegars with bentonite, kieselghur or gelatin for 1-2 days, depending on temperature applied. Decantation process is followed by filtration procedure. Filtration is carried out with 0.2 μ cross-flow microfiltration for a couple hours. The end product is called "filtered vinegar" that contains 10% acid and could be sold as is or goes through packaged production. At packaged production stage acidity of vinegar is adjusted to 4% with water. Afterwards, antioxidants (sulphur dioxide or sodium metabisulfite) are added. After all these steps vinegar products are ready to market.

3.1.2 Production Process of Nahita Natural Fermentation Vinegars and Yedier Vinegars

Corresponding to the information provided by Nahita, first step in vinegar processing is procuring of the fruit. Fruit is washed and cut into pieces with a shredding machine. Shredded fruit pieces are taken into tanks to release their water. After sending away pulp and dehydrated mash it is left for ethyl alcohol production. Ethyl alcohol production is dependent on whether conditions and this process can take 2-6 weeks. After starting ethyl alcohol fermentation fruit ethanol is left for 1-3 months. At the end of alcohol fermentation, fruit mash is taken into acetic acid fermentation step. Old vinegar is added to initialize the acetic acid fermentation and this duration can take 2-5 months depending on whether conditions. After this step, vinegar is left to rest for at least 3 months and this way vinegar production is accomplished.

3.2 Chemicals

Methanol ($\geq 99.9\%$), formic acid ($\geq 98\%$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), hydrochloric acid (37%), sodium acetate trihydrate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) and trifluoroacetic acid (99%) were obtained from Merck KGaA (Darmstadt, Germany). Gallic acid ($\geq 98\%$), Folin-Ciocalteu's phenol reagent, ethanol ($\geq 99.8\%$), pepsin enzyme, pancreatin enzyme, bile salts, acetonitrile (99.8%) and sodium bicarbonate were taken from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), potassium chloride (KCl) was obtained from Riedel-de Haen Laborchemikalien GmbH (Hanover, Germany).

3.3 Equipments

The equipments used in this study were Memmert water bath, SHIMADZU UV-Visible spectrophotometer, SP-3000 Nano spectrophotometer, IKA Vorteks Genius 3, IKA Werke agitator, Precisa XB 220A balance, Hettich Zentrifugen Universal 32 R refrigerated centrifuge, HANNA HI 2211-02 pH meter, Waters W600 HPLC system with PDA (Waters 996) detector, Luna C18 column (Phenomenex).

3.4 Methods

3.4.1 Dry Matter Content

Dry matter content of the vinegar samples was measured to determine process effect on antioxidative activity of vinegars. Abbe refractometer was used for this purpose.

3.4.2 Extract Preparation

Grapes and grape vinegars could contain many substances, such as sugars and maillard reaction products may be present in traditional balsamic vinegar, and may interfere with the assessment results. Therefore, some researchers used Sephadex C-18 columns to separate the phenols from those compounds and investigated polyphenols, polymeric tannins and Maillard reaction products (Verzelloni et al., 2007 and Verzelloni et al., 2009). However, in this study, it was aimed to investigate total flavonoid content, total phenolic content and antioxidant capacity of ready to use vinegar samples therefore any extraction procedure was not applied to vinegar

samples. All samples were centrifuged at 4000 rpm for 4 minutes to eliminate the turbidity elements.

3.4.1 *In Vitro* Bioaccessibility Method

In vitro bioaccessibility method was adapted from a study of McDougall et al. (2005). The preparation steps include firstly 0.05 g pepsin adjustment with 50 mL of 0.1N of HCl. Approximately 37.5 mL of this solvent was taken into a flask and 1g NaCl was added and total volume was adjusted to 500 mL with MQ water in order to prepare stomach solvent. For preparing small intestinal media, 10.5 g of NaHCO₃ was adjusted 250 mL with MQ water. 20 mL of this solution was taken into a dialysis bag of 20 cm length and its both ends were connected. Finally, 0.1 g of pancreatin and 0.625 g of bile salt were dissolved in 25 mL MQ water separately and then mixed with each other. Approximately 5 mL of samples were taken into 250 mL beaker. Total volume was adjusted 20 mL with stomach solution. Mixture was shaken for homogenous dispersion for 10 seconds with agitator and pH is set to 2.0±0.5 with 5 N HCl. Sample was placed into shaker water bath for 2 hours at 37°C and at the end of this period 2 mL of solvent was taken from the beaker as "Post Gastric Solutin (PG)" and the dialysis bag was put into beaker, 4.5 mL of pancreatin-bile salt mixture was added into the sample. Samples placed into shaker water bath for 2 hours at 37°C again. After this step, mixture inside the dialysis bag was called as "IN fraction", which remained part of the sample at the intestine, and mixture outside the dialysis bag was called as "OUT fraction", which remained part of the sample out the intestine. Those fractions were taken into eppendorph tubes and centrifuged at 18000 rpm, 4°C. Total flavonoid content, total phenolic content and antioxidant activity were examined on obtained fractions.

3.4.1 Total Flavonoid Content

Total flavonoid content was determined based on the method of Dewanto et al. (2002) as spectrophotometrically. In brief, 250 µL of sample was taken into an analysis tube, 1.25 mL MQ water was added to sample. Afterwards, 75 µL of 5% NaNO₂ solvent was added and the mixture was kept for 6 minutes, then 150 µL of 10% AlCl₃.6H₂O solvent was added. After waiting for 5 minutes, 0.5 mL of 1 M NaOH solvent was added and total volume was adjusted to 2.5 mL with MQ water. Absorbance was measured at 510 nm wavelength against a blank. Experiments were

conducted in triplicate and mean values were reported. Catechin in 75% MeOH was used for generating the standard curve. Standard calibration curve for total flavonoid content analysis is shown in Figure A. 1.

3.4.2 Total Phenolic Content

Total phenolic content of samples measured based on Folin-Ciocalteu method (Spanos and Wrolstad, 1990). Briefly, 100 μ L of sample was put into an analysis tube and 900 μ L water was added. Subsequently 5 mL of 0.2 N of Folin-Ciocalteu reagent was added and for the mixture was kept for 3 minutes. Then, 4 mL of saturated Na_2CO_3 solvent was added and the mixture was kept for 90 minutes. At the end of this period absorbance was measured at 765 nm wavelength against a blank. Gallic acid in 75% MeOH was used for generating the standard curve. Standard calibration curve for total phenolic content analysis is shown in Figure A. 2.

3.4.3 Total Antioxidant Capacity

Measurement of total antioxidant capacity of vinegar samples were performed using 4 different methods which are generally used for fruits and vegetables. Experiments were conducted in triplicate and mean values were reported. Trolox in 75% MeOH was used for the standard curve.

3.4.3.1 ABTS Method

ABTS method used was based on Miller ve Rice-Evans (1997). Briefly, 220 mg of ABTS was dissolved in 200 mL of MQ water and 38 mg of $\text{K}_2\text{S}_2\text{O}_8$ was dissolved in 2 mL of MQ water. These solutions were mixed and stored overnight in the dark to complete the radicalization. After this process, ABTS⁺ solution was obtained. ABTS⁺ solution was diluted with 0.05 M KPi buffer (pH=8) until its absorbance reached 0.9 ± 0.2 . Approximately 100 μ L of sample was taken into an analysis tube and 1 mL of ABTS⁺ solution was added to sample. with the mixture was vortexed for 15 seconds. Absorbance was measured after 45 seconds at 734 nm wavelength against MQ water. Standard calibration curve of TROLOX for ABTS analysis is shown in Figure A. 3.

3.4.3.2 CUPRAC Method

CUPRAC method was based on study of Apak et al. (2004). Briefly, 0.4262 g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 250 mL of MQ water, 19.27 g of NH_4Ac was diluted in 250 mL of MQ water, and 0.039 g of Neocuproine was dissolved in 96% EtOH and diluted to 25 mL. Approximately 100 μL of sample was taken into an analysis tube and 1 mL of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ solvent, 1 mL of Neocuproine, 1 mL of NH_4Ac buffer and 1 mL of MQ water were added sequentially. After keeping the mixture for 30 minutes, absorbance was measured at 450 nm wavelength against a blank. Standard calibration curve of TROLOX for CUPRAC analysis is shown in Figure A. 4.

3.4.3.3 DPPH Method

DPPH method was based on Kumaran et al. (2006). In brief, 2 mL of 0.1 mM DPPH was mixed with 100 μL of sample in a test tube. Samples were stored in dark at room temperature for 30 minutes. Absorbance was measured at 517 nm wavelength against methanol. Standard calibration curve of TROLOX for DPPH analysis is shown in Figure A. 5

3.4.3.4 FRAP Method

FRAP method was adapted from the study of Benzie and Strain (1996). Briefly, 3.1 g of $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ was dissolved in MQ water, 16 mL of 99.85% acetic acid was added and total volume was adjusted to 1 L with MQ water. Approximately 0.504 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in MQ water and mixed with 1M of 37% HCl. Total volume of the mixture was adjusted to 100 mL with MQ water. Approximately 0.156 g TPTZ was dissolved in 50 mL of EtOH. FRAP reagent was prepared with 10:1:1 volume ratio with these solution sequence. Afterwards, 100 μL of sample was taken into a test tube and 900 μL of FRAP reagent was added. After keeping the mixture for 4 minutes at room temperature absorbance was measured at 593 nm wavelength against MQ water. Standard calibration curve of TROLOX for FRAP analysis is shown in Figure A. 6.

3.4.4 HPLC Analysis of Vinegar Phenolic Profile

Phenolic profiles of all samples were also evaluated by HPLC-PDA for vinegar processing, bioaccessibility of vinegars -with initial values and, PG, IN and OUT

fractions- and different types of vinegars. As a consequence of several authors generally agreed with that most vinegar substitutes contain similar phenolic compounds such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, (+)-catechin, syringic acid, caffeic acid, *p*-coumaric acid, specific phenolics were determined in this study (Que et al., 2009; Matejíček et al., 2005; Samanidou et al., 2001; Sagdic et al., 2011). HPLC-PDA results of samples were given as mg /100 mL samples for all.

HPLC analysis were carried out by using the method adapted from Capanoglu et al. (2008). Standard calibration curves were prepared by using gallic acid, protocatechuic acid, pHBA(-*P*-hydroxy benzoic acid), caffeic acid, vanilic acid, catechin, *p*-coumaric acid, syringic acid. These samples and stock solutions were filtered through a 0.45- μ m membrane filter and 1 ml of the filtered sample was placed into vials and analyzed in a Waters W600 HPLC system with PDA (Waters 996) detector, for each sample. Luna C18 column (Phenomenex) was used as the stationary phase.

The mobile phase was including solvent A, Milli-Q water with 0.1% (v/v) TFA and solvent B, acetonitrile with 0.1% (v/v) TFA, acetonitrile with 0.1% (v/v) TFA. A Linear gradient was used as follows: at 0 min, 95% solvent A and 5% solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min returns to initial conditions. The flow rate was 1 ml/min. Detections were done at 280, 312, 360, and 520 nm wavelengths. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard curves. Standard calibration curves of HPLC-PDA analysis are shown in Appendix A and Chromatograms of all samples were shared at Appendix B.

3.4.5 Statistical Analyses

The results were analyzed by IBM SPSS Statistics Program (21th version) by using one way analysis of variance (ANOVA) at 0.05 significant level and Tukey's New Multiple Range Test was applied as post hoc tests. The differences between all samples, PG, IN and OUT fractions were evaluated statistically. Tukey's Range Test was applied to exact values to observe the differences between total phenolic and flavonoid contents, and antioxidant activity ($p < 0.05$). Each analysis was performed

in triplicate and the results were reported as mean value $\pm$ standart deviation. Statistical analysis results of samples is given at Appendix C.

4. RESULTS AND DISCUSSION

The main goal of this research was to investigate the effects of vinegar processing on grape and apple fruit antioxidants. For this purpose, samples were collected from different steps of grape and apple vinegar processing and monitored for the changes in total flavonoid content (TFC), total phenolic content (TPC) and total antioxidant capacity (TAC). In addition, four different types of vinegars, including grape, apple, pomegranate, and balsamic vinegars, were subjected to *in vitro* simulated gastrointestinal (GI) digestion protocol in order to determine and compare the *in vitro* bioaccessibility of these fruit antioxidants in processed vinegar samples. Moreover, various vinegar samples were evaluated and compared for their TFC, TPC, and TAC values.

4.1 Dry Matter Content

Dry matter contents of grape and apple vinegar processing samples were measured for the clear assesment of the effects of processing fruit antioxidants on dry weight basis. Samples from Kuhne Vinegar Factory were selected for this purpose. Dry matter contents of grape and apple vinegar processing samples are given in Table 4.1.

Table 4.1: Dry matter content of the samples from each processing step.

<i>Apple Vinegar Process Steps</i>		<i>Grape Vinegar Process Steps</i>	
Apple Juice Concentrate	70.50±0°	Grape Wine	7.50±0°
Apple Wine	7.50±0°	Not Decanted Not Filtered Grape Vinegar	8.25±0°
Not Filtered Apple Vinegar	8.8±0.12°	Decanted Not Filtered Grape Vinegar	7.8 ±0.12°
Filtered Apple Vinegar	7.8±0.12°	Filtered Grape Vinegar	7.7±0.5°
Apple Vinegar	4.3±0.4°	Grape Vinegar	3.75±0.3°

4.2 The Effects of Vinegar Processing on Antioxidant Compounds

Grape vinegar and apple vinegar were utilized for measuring the process effect on total flavonoid-total phenolic content and antioxidant capacity of samples. Results are given as fresh weight basis (FWB)and dry weight basis (DWB) at tables.

4.2.1 Effects of Vinegar Processing on Grape Antioxidants

The grape vinegar processing was studied for its effects on TPC, TFC, and TAC of different processing samples including grape wine (GW), raw grape vinegar (not decanted and not filtered) (RGV), decanted grape vinegar (DGV), decanted and filtered grape vinegar (DFGV), and final grape vinegar (FGV) samples.

4.2.1.1 Changes in Total Phenolic and Total Flavonoid Contents

The TFC and TPC values, determined for grape vinegar processing samples, are represented in Table 4.2. The TFC and TPC results are expressed in mg gallic acid equivalent (GAE)/100 mL sample and mg catechin equivalent/100 mL sample for FWB, mg GAE/100 g sample and mg catechin equivalent/100 g sample for DWB, respectively. The comparisons on fw basis revealed significant decreases in TFC and TPC from grape wine to grape vinegar ($p<0.05$), whereas these samples were not found to be significantly different regarding to their TFC and TPC when the calculations were performed on dw basis ($p>0.05$). On the other hand, samples collected from the intermediary steps of processing were all found to be significantly lower in their TFC and TPC values, in comparison to grape wine sample, both on FWB and DWB calculations (Table 4.2.).

Table 4.2: Total flavonoid and phenolic contents of samples from grape vinegar production steps on fresh weight basis and dry weight basis.

	FWB		DWB	
	TFC*	TPC**	TFC*	TPC**
GW	10.86±0.54 ^a	68.86±10.76 ^a	144.83±7.25 ^a	918.07±143.51 ^a
RGW	7.36±0.96 ^b	55.80±5.69 ^b	79.32±30.13 ^b	676.34±68.95 ^b
DGV	6.34±1.12 ^b	38.57±2.07 ^c	80.89±14.30 ^b	492.48±27.25 ^c
DFGV	7.76±1.42 ^b	38.63±1.55 ^c	100.93±15.87 ^b	505.81±36.74 ^c
FGV	5.83±0.63 ^c	31.66±4.34 ^c	153.26±18.78 ^a	841.65±170.66 ^a

* : mg CA/100 mL samples at FWB and mg CA/100 mg samples at DWB.

**:.mg GAE/100 mL samples at FWB and mg GAE/100 mg samples at DWB.

Grape wine as GW, raw grape vinegar (not decanted and not filtered) as RGV, decanted grape vinegar as DGV, decanted and filtered grape vinegar as DFGV, and final grape vinegar as FGV.

The grape juice concentrate processing was reported to lead to 87.8% and 84.4% losses in TFC and TPC, respectively. While the TPC of the starting fruit was found to be 1619 ± 167 mg GAE/100 g DWB, it was 323 ± 48 mg GAE/100 g dwb after pasteurization and 252 ± 33 mg GAE/100 g DWB in grape juice concentrate (Capanoglu et al., 2013). Elimination of the seed and skin and also the pasteurization step are the possible reasons of occurred changes at TFE and TPC values.

Gollücke et al. (2009), inquired the evolution of major phenolic components and radical scavenging activity of grape juices through concentration process and storage. Concentrated grape juice samples of Concord and Isabel cultivars were used in this study. Hot pressing of grapes, pasteurization of must, filtration and concentration of juice steps were investigated/studied. According the results total phenolic content varied from 2872.9 to 2587.6 GAE in Concord Juice and from 1756.8 to 1428.9 GAE in Isabel Juice and the mean

TP retention percentage was 90% and 81% for Concord Juice and Isabel Juice, respectively. This study confirms the results mentioned in previous research.

In another study, the changes in TPC and TAC were monitored throughout a wine making process, with the processing samples including grape, pomace, juice, must, and wine. It was pointed out that although TPC did not much differ during processing, TAC was found to be enhanced after fermentation step (Yildirim et al., 2005). According to results, TPC values of red grape and white grape varied between 2850 ± 955.5 and 442.5 ± 43.13 , pomace was 1211 ± 225.1 , red juice was 1450 ± 838.2 , must was 2750 ± 948.1 , and also red wine and white wine were 1787 ± 835.0 and 406.9 ± 87.7 mg GAE/L, respectively.

Da'valos et al. (2005), who investigated the antioxidant properties of red grape juice, white grape juice, and wine vinegar, attributed the differences between these products to their varying phenolic contents and compositions, as well as to the other non-phenolic antioxidants present in the samples. Red grape juice was reported to have the highest TPC (705 – 1177 mg GAE/L) in this study, which was followed by wine vinegar (637-867 mg GAE/L) and white grape juice (151-474 mg GAE/L), respectively. Wine vinegar TPC results obtained from red grape juice were fit with grape wine samples in our study used for investigation of effects of vinegar processing on grape antioxidants.

In the light of this information, a significant difference was observed at fermentation step from grape wine to grape vinegar in TFC and TPC analysis with FWB, although considering the DWB of samples indicated that fermentation stage of wine has no significant effect on vinegars.

4.2.1.2 Changes in Antioxidant Capacity

Antioxidant capacity of the samples were measured with ABTS, CUPRAC, DPPH, and FRAP tests and are given together with FWB at Table 4.3 and DWB at Table 4.4. Results are given mg TEAC/100 mL sample at FWB and mg TEAC/100 g sample at DWB for all antioxidant capacity assay, additionally significant difference was determined between the samples ($p < 0.05$).

Table 4.3: Antioxidant capacity of samples from grape vinegar production steps on FWB.

	ABTS*	CUPRAC*	DPPH*	FRAP*
GW	112.11±24.62 ^a	118.29±13.63 ^a	105.72±1.88 ^a	32.45±1.79 ^a
RGV	87.61±19.29 ^b	90.32±18.85 ^b	74.37±2.59 ^b	28.17±1.12 ^b
DGV	32.74±4.03 ^c	80.17±7.45 ^b	68.20±3.34 ^c	22.93±1.59 ^c
DFGV	33.70±2.64 ^c	79.45±10.21 ^b	67.94±2.54 ^c	23.03±0.88 ^c
FGV	43.89±7.06 ^{bc}	76.11±10.33 ^b	60.84±3.88 ^d	21.56±2.41 ^c

*: mg TEAC/100 mL at FWB.

Grape wine as GW, raw grape vinegar (not decanted and not filtered) as RGV, decanted grape vinegar as DGV, decanted and filtered grape vinegar as DFGV, and final grape vinegar as FGV.

Results indicated there is a significant decrease of ABTS values from 112.11±24.62 to 43.89±7.06, CUPRAC values from 118.29±13.63 to 76.11±10.33 DPPH values from 105.72±1.88 to 60.84±3.88 and FRAP values from 32.45±1.79 to 21.56±2.41 mg TEAC/100 mL as seen at table. 4.3. This situation observed vary considering the results of dry content based table 4.4. There is also a decrease in antioxidant capacity in ABTS essay on DWB, however, there is an increase about 29.9% for CUPRAC, 14.4% for DPPH, and 31.3% for FRAP essays.

When the results were examined for each successive step, the values observed for DGV, DFGV were found not to be statistically different. Furthermore, although there was a dilution step included in processing of decanted and filtered vinegar samples to the final vinegars, the values obtained for the samples from these different steps were found to be similar to each other on DWB, differentiating from what was expected. This could be explained with the up-regulation of the antioxidant levels in the final product with the additional use of sodium metabisulfite as an antioxidant. Several researchers agreed with sodium metabisulfite may be designated for acidic preparations and used as antioxidants for vitamin C to reduce the vitamin C degradation (Gunnison and Jacobsen, 1987; Wade and Weller, 1994; Touitou et al., 1996; Karg et al., 1987).

Table 4.4: Antioxidant capacity of samples from grape vinegar production steps on DWB.

	ABTS*	CUPRAC*	DPPH*	FRAP*
GW	1494.75±328.27 ^a	1577.19±181.67 ^b	1409.67±25.11 ^b	432.61±23.84 ^b
RGV	1061.95±233.84 ^a	1137.51±205.76 ^c	901.50±31.39 ^c	341.49±13.62 ^c
DGV	417.70±49.40 ^b	1024.03±99.38 ^c	870.72±43.82 ^c	292.93±22.94 ^c
DFGV	440.69±36.03 ^b	1042.33±163.73 ^c	888.14±39.33 ^c	302.05±28.83 ^c
FGV	1158.21±206.67 ^{ab}	2048.42±191.46 ^a	1612.82±244.45 ^a	568.10±75.91 ^a

*: mg TEAC/100 mg samples at DWB.

Grape wine as GW, raw grape vinegar (not decanted and not filtered) as RGV, decanted grape vinegar as DGV, decanted and filtered grape vinegar as DFGV, and final grape vinegar as FGV.

The total antioxidant capacity results showed the same tendency over the four different methods in the study performed by Capanoglu et al. (2013). According to the results concentration process resulted in a reduction in antioxidant capacity by 83–92% from grape to concentrate. The range of antioxidant capacity of grape to concentrate was changed from 3188±373 to 456±42, from 4960±512 to 843±285, from 6910±421 to 527±28, and from 1281±47 to 188±6 $\mu\text{mol TEAC}/100\text{ g}$ of dry weight for ABTS, CUPRAC, DPPH and FRAP assays. These results clearly indicated that grape concentrating process had a reduction effect on antioxidant capacity, however in the case of investigating the process step by step, it is obvious to say pasteurization stage is the main reason of this loss according the statistical results of the research.

Gollücke et al. (2009), in the continuation of their study, appraised the radical scavenging capacity of the Concord juice and Isabel juice with DPPH analysis. Radical scavenging capacity of Concord juice and Isabel juice was reported to decrease from 9.68 to 7.45 mM TE and from 7.40 to 6.33 mM TE. These values indicate 77% retention of antioxidants for Concord juice and 86% retention for Isabel juice at DPPH analysis.

It was reported that Cabernet Sauvignon red wine showed a free-radical scavenging activity equivalent to that of a Trolox solution 6 mM with the ABTS method, 7.5 mM with the DPPH method, and 9.4 mM with the FRAP method (Gil et al., 2000).

In a study by Cerezo et al., (2010) anthocyanin composition in Cabernet Sauvignon red wine vinegar obtained by submerged acetification was determined. Antioxidant activity of wine and vinegar was determined as 11.23 ± 0.11 and 9.61 ± 0.20 mM TEAC/g sample extract for FRAP assay, 12.26 ± 0.18 and 10.2 ± 0.3 mM TEAC/g sample extract for DPPH assay. A decrease in antioxidant values from wine to vinegar by 14.4%, 16.8% for FRAP and DPPH tests was observed.

In the case of cross checking the loss percentage of antioxidant values from wine to vinegar, the losses observed in our research are higher than the values reported in the study of Cerezo et al. (2010). This difference is possibly due to utilization of different grape types/varieties and processing techniques.

4.2.2 Effects of Vinegar Processing on Apple Antioxidants

The effects of apple vinegar processing on TPC, TFC, and TAC of several processing samples was studied. Samples include apple juice concentrate (AJC), apple wine (AW), decanted apple vinegar (DAV), decanted and filtered apple vinegar (DAV), and final apple vinegar product (FAV).

4.2.2.1 Changes in Total Phenolic and Total Flavonoid Contents

Total flavonoid content and total phenolic content of the samples are given together with either fresh or dry weight based at Table 4.5. TPC and TFC results are expressed in mg gallic acid equivalent (GAE)/100 mL sample and mg catechin equivalent/100 mL sample for FWB, mg GAE/100 g sample and mg catechin equivalent/100 g sample for DWB, respectively. Besides, significant difference was determined between the samples ($p < 0.05$).

A significant decrease in total flavonoid and phenolic contents of samples from apple juice concentrate to apple vinegar at FWB was observed similar to grape vinegar production steps. Comparing the results at DWB 94.6% and 88.1% recession of total flavonoid content. However, total phenolic content at FWB give place to 9.6% drop of total flavonoid content

and 97.7% increment of total phenolic content at DWB from apple concentrated to apple vinegar.

Table 4.5: Total flavonoid and phenolic contents of samples from apple vinegar production steps on FWB and DWB.

	FWB		DWB	
	TFC*	TPC**	TFC*	TPC**
AJC	32.86±3.08 ^a	163.53±13.65 ^a	46.60±4.37 ^c	231.95±19.35 ^d
AW	18.01±0.74 ^b	38.93±4.12 ^b	240.16±9.89 ^a	519.04±54.95 ^a
DAV	7.14±1.05 ^c	33.82±1.22 ^{bc}	80.77±12.34 ^b	382.84±12.27 ^c
DFAV	5.06±1.16 ^c	27.98±1.76 ^{cd}	64.36±14.30 ^b	357.17±21.48 ^c
FAV	1.78±0.22 ^d	19.49±1.69 ^d	42.13±8.55 ^c	458.63±58.45 ^b

* : mg CA/100 mL samples at FWB and mg CA/100 mg samples at DWB.

**::mg GAE/100 mL samples at FWB and mg GAE/100 mg samples at DWB.

Apple juice concentrate as AJC, apple wine as AW, decanted apple vinegar as DAV, decanted and filtered apple vinegar as DFAV, and final apple vinegar product as FAV.

The influence of apple variety on the polyphenol profile of apple wines were investigated by Satore et al. (2008), and polyphenols content of samples were found between 228.0±14.8 and 639.0±28.8 mg GAE/L. In addition to this antioxidant activity was measured as 88.7±4.2 and 466.1±6.0 mg TE/100 mL in ABTS test.

These findings are in accordance with our results obtained for apple wine. a sharp decrease at fermentation steps from AJC to FAV was observed. On the other hand it is visible that ethyl alcohol fermentation resulted in an increase in TFC and TPC values of AJC, on DWB.

4.2.2.2 Changes in Antioxidant Capacity

Antioxidant capacity of the samples were measured with ABTS, CUPRAC, DPPH and, FRAP analysis and are given on FWB in Table 4.6 and on DWB in Table 4.7. Results are given mg TE/100 mL samples for all antioxidant capacity essays, significant difference was determined between the samples (p<0.05).

Table 4.6: Antioxidant capacity of samples from apple vinegar production steps on FWB.

	ABTS*	CUPRAC*	DPPH*	FRAP*
AJC	1805.60±183.32 ^a	585.52±74.52 ^a	1036.28±52.39 ^a	226.36±14.04 ^a
AW	177.90±16.79 ^b	120.12±9.31 ^b	117.81±8.30 ^b	40.27±1.21 ^b
DAV	50.37±4.27 ^c	57.25±9.11 ^c	62.92±2.74 ^c	22.67±1.56 ^c
DFAV	45.95±1.86 ^c	44.22±8.40 ^c	55.92±1.82 ^c	19.86±0.80 ^{cd}
FAV	53.78±6.51 ^c	43.97±10.97 ^c	46.01±2.14 ^c	13.90±2.19 ^d

*: mg TEAC/100 mL at FWB.

Apple juice concentrate as AJC, apple wine as AW, decanted apple vinegar as DAV, decanted and filtered apple vinegar as DFAV, and final apple vinegar product as FAV.

Results showed that there are loss of antioxidant capacity, 97.0% for ABTS, 92.5% for CUPRAC, 95.6 for DPPH, and 93.8% for FRAP, at apple vinegar comparing with concentrated apple juice. Considering the dry content based table these rate changes into decrease for ABTS and DPPH, 50.9% and 26.0%, respectively. However, it leads to an increase of 9.4% and 0.7% for CUPRAC and FRAP essays, respectively.

Table 4.7: Antioxidant capacity of samples from apple vinegar production steps on DWB.

	ABTS*	CUPRAC*	DPPH*	FRAP*
AJC	2561.14±260.03 ^a	830.52±105.70 ^b	1469.91±74.31 ^a	321.07±19.92 ^b
AW	2372.06±223.83 ^a	1601.64±124.15 ^a	1570.85±110.69 ^a	536.95±16.14 ^a
DAV	570.34±48.97 ^c	648.48±104.55 ^c	712.23±28.48 ^c	256.53±16.08 ^c
DFAV	586.53±20.82 ^c	565.28±111.42 ^c	713.90±22.06 ^c	253.50±8.27 ^c
FAV	1256.48±110.25 ^b	908.40±118.21 ^b	1087.04±148.92 ^b	323.48±31.22 ^b

*: mg TEAC/100 mg at DWB.

Apple juice concentrate as AJC, apple wine as AW, decanted apple vinegar as DAV, decanted and filtered apple vinegar as DFAV, and final apple vinegar product as FAV.

The total antioxidant activity of apple juice was investigated in a study by Miller & Rice-Evans (1997). TEAC values of apple juice was reported to be $840 \pm 50 \mu\text{mol/l}$ based on ABTS test.

Antioxidant capacity and polyphenolic content of different apple extracts varied amongst 780 ± 44 and $1.421 \pm 45 \mu\text{mol TE/100 g}$ for FRAP assay, 99 ± 2 and $176 \pm 3 \text{ mg GAE/100 g}$ for total phenolic content assay (Lotito and Frei, 2004a).

Our AJC result is higher than the result reported by Miller & Rice-Evans (1997) for ABTS analyze, nevertheless FRAP results were significantly lower than the values reported by Lotito and Frei (2004a). This difference is possibly due to utilization of apple extracts and AJC for tests. As mentioned earlier, pasteurization, a production step in juice concentrate, caused sharp drops in TPC and TAC values.

4.3 The Effects of Vinegar Processing on Phenolic Profile

Grape vinegar and apple vinegar were utilized for measuring the process effect on phenolic compounds of samples just happened at while measuring the total flavonoid-total phenolic content and antioxidant capacity of samples.

4.3.1 Effects of Vinegar Processing on Grape Phenolics

The grape vinegar processing was studied for its effects on phenolic compounds of different processing samples including grape wine (GW), raw grape vinegar (not decanted and not filtered) (RGV), decanted grape vinegar (DGV), decanted and filtered grape vinegar (DFGV), and final grape vinegar (FGV) samples. Phenolic compounds of the samples were measured with HPLC-PDA and gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.8 and results of samples were given as mg /100 mL samples.

Table 4.8: Effects of vinegar processing on grape phenolics at FWB.

	Gallic Acid	<i>p</i>-Hydroxybenzoic Acid
GW	67.48±24.18	N.D.
RGV	63.33±0.31	N.D.
DGV	5.08±1.64	1.06±0.53
DFGV	5.14±1.62	1.19±0.74
FGV	6.41±1.50	0.90±0.05

*Results were given in mg /100 mL samples for all.

**N.D. : Not detected.

***Grape wine as GW, raw grape vinegar (not decanted and not filtered) as RGV, decanted grape vinegar as DGV, decanted and filtered grape vinegar as DFGV, and final grape vinegar as FGV.

Obtained results by HPLC-PDA indicated that GW is rich in gallic acid, however *p*-hydroxybenzoic acid was not detected in this sample as happened in RGV sample. After decantation process gallic acid concentration sharply decreased to 5.08±1.64 mg/100 mL and in addition to this *p*-hydroxybenzoic acid was started to be seen in samples.

Hydroxycinnamic acids, namely *p*-coumaric acid, caffeic acid, and ferulic acid are most common phenolic acids in grapes, however process steps of product could be affected after hydrolysis and they may not be observed at beginning and hydroxybenzoic acids are mostly represented in grapes by gallic acid and degradation of flavonoids may cause augmentation at other phenolics (Cheynier et al., 2010).

4.3.2 Effects of Vinegar Processing on Apple Phenolics

The effects of apple vinegar processing on phenolic compounds of several processing samples were studied. Samples include apple juice concentrate (AJC), apple wine (AW), decanted apple vinegar (DAV), decanted and filtered apple vinegar (DAV), and final apple vinegar product (FAV). Phenolic compounds of the samples were measured with HPLC-PDA and gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.9 and results of samples were given as mg /100 mL samples.

Table 4.9: Effects of vinegar processing on apple phenolics at FWB.

	Gallic Acid	<i>p</i>-Hydroxybenzoic Acid	Catechin	Syringic Acid	Caffeic Acid	<i>p</i>-Coumaric Acid
AJC	1.01±0.05	1.80±0.11	70.30±4.90	5.24±0.27	10.27±0.10	2.37±0.00
AW	0.22±0.02	0.20±0.02	16.18±0.25	0.48±0.03	2.32±0.05	0.26±0.00
DAV	1.78±0.01	1.08±0.26	7.36±0.20	0.38±0.09	1.12±0.04	0.20±0.02
DFAV	5.16±0.01	1.17±0.04	2.79±0.31	0.20±0.00	0.52±0.01	0.11±0.01
FAV	0.81±0.35	0.16±0.09	2.37±0.13	0.12±0.02	0.36±0.00	0.08±0.01

* Results were given in mg/100 mL samples for all.

**N.D. : Not detected.

***Apple juice concentrate as AJC, apple wine as AW, decanted apple vinegar as DAV, decanted and filtered apple vinegar as DFAV, and final apple vinegar product as FAV.

At the beginning of the process gallic acid, *p*-hydroxybenzoic acid, catechin, syringic acid, caffeic acid, *p*-coumaric acid were all found in the AJC, but protocatechuic acid. Most remarkable value was observed at catechin concentration at AJC with 70.30±4.90 mg/100 mL at FWB. After alcoholic fermentation step there were decrease at gallic acid, *p*-hydroxybenzoic acid, catechin, syringic acid, caffeic acid, *p*-coumaric acid concentration, in contrast to protocatechuic acid.

In a study performed by Satora et al. (2008), the antioxidant activity and polyphenols profile of wines produced from different apple varieties were investigated and *p*-coumaric acid concentration found 0.1±0.0-0.9±0.2 mg/100 mL of apple wine, catechin concentration found 1.6±0.4-3.5±0.6 mg/100 mL of apple wine.

4.4 The Effects of *In Vitro* Bioaccessibility on Vinegar Antioxidants

The *in vitro* bioaccessibility of vinegar samples were also investigated in this study because of the lack of information about vinegar digestion in the literature. For this purpose aforementioned grape vinegar, apple vinegar, pomegranate vinegar and, balsamic vinegar were examined. The *in vitro* bioaccessibility method was applied to vinegars directly and all analysis -TFC, TPC and TAC- were performed on PG, IN and OUT fraction of vinegars.

4.4.1 The Effects of *In Vitro* Bioaccessibility on Grape Vinegar

Grape vinegar, supplied by Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions were analyzed. Furthermore, IN fraction was checked against initial value of grape vinegar and this comparison amount was reported in percent at "Recovery" section.

4.4.1.1 Changes in Total Phenolic and Total Flavonoid Contents

TFC and TPC of the initial value, and PG, IN, OUT fractions of grape vinegar are given in Table 4.10 on FWB. Results are given mg CA/100 mL samples for TFC and mg GAE/100 mL samples for TPC. Significant difference was determined between the samples ($p < 0.05$). PG fraction of the grape vinegar sample had the highest flavonoid and phenolic content whereas IN fraction had the lowest amount among the bioaccessibility fractions. Recovery percentage for phenolics were found 83.6%, meantime flavonoids were 37.1%.

Table 4.10: Total flavonoid and phenolic contents of grape vinegar and *in vitro* digested grape vinegar samples.

Grape Vinegar		TFC*	TPC**
	INITIAL	5.52±0.97 ^a	26.38±1.29 ^a
	PG	4.64±0.17 ^b	25.49±4.09 ^a
	IN	2.05±0.07 ^c	22.06±4.54 ^a
	OUT	4.03±0.18 ^c	22.34±3.20 ^a
	RECOVERY %	37.1	83.6

* : mg CA/100 mL samples

** : mg GAE/100 mL samples

García-Alonso et al. (2006), investigated the effect of the acute intake of phenolic-rich juice on antioxidant status in healthy subjects. Water mixed commercially available concentrated juices of grape (26%), cherry (2%), blackberry (0.6%), blackcurrant (0.6%), and raspberry (1%) were utilized for the research. Increments were calculated for each subject by subtracting its baseline level from each post-intake value. They observed no significant in the mean values of serum FRAP, uric acid, vitamin C, or serum lipid-bound polyphenols after the intake of the juice or during the study. However comparing each FRAP values of volunteer with individual's baseline level resulted in a significant difference in serum FRAP. Authors explained this situation as the lack of statistical significance which can be attributed to the

high interindividual variations observed for these parameters. The serum levels of lipid-bound polyphenols were reported to show a mean value of around 230 mg GAE/L, with no significant differences during the time of the study even then, serum lipid-bound polyphenols slightly increased 2 hours post intake and this effect proceeded for the duration of the experiment.

In another study, gastric and pancreatic digestion of grape phenols were measured by *in vitro* bioaccessibility assay (Tagliazucchi et al., 2010). After simulated mastication corresponding to zero time of *in vitro* gastric digestion, the bio-accessible total polyphenols were 34.88 mg of catechin/100 g of grapes corresponding to 49.7% of the total polyphenols and total flavonoids were 8.80 mg of catechin/100 g of grapes to 27.2% of the total flavonoids. Statistically there were no significant difference between bioaccessibility of total phenols and flavonoids. The TPC recovery amount of grape vinegar is bigger than grape phenols, despite of this flavanoid recovery of grape vinegar was lower.

In a study by Modun et al. (2008), the increase in human plasma antioxidant capacity after red wine consumption was investigated with *in vivo* assay. Red wine (RW) and dealcoholized red wine (DARW) samples were prepared for this purpose. Total phenolic content of DARW and RW was reported as 2790 ± 20 GAE mg/L and 2800 ± 25 GAE mg/L, respectively.

4.4.1.2 Changes in Antioxidants Capacity

Antioxidant capacity of the Total PG, IN and OUT fractions of grape vinegar are given in Table 4.11 on FWB. Fractions were tested using ABTS, CUPRAC, DPPH and, FRAP assays. Results are given in mg TEAC/100 mL samples for all assays, additionally significant difference was determined between the samples ($p < 0.05$). Except DPPH assay, it was observed that more than half of initial antioxidant capacity passed through PG fraction. Values for IN fraction were 5.94 ± 1.28 for ABTS, 23.06 ± 3.06 for CUPRAC, 5.25 ± 1.11 for DPPH and 5.24 ± 1.00 for FRAP analyzes, and these refer to 10.6%, 39.6%, 18.5% and 12.5% recovery percentage.

Table 4.11: Antioxidant capacity of grape vinegar and *in vitro* digested grape vinegar samples.

		ABTS*	CUPRAC*	DPPH*	FRAP*
Grape Vinegar	INITIAL	55.88±6.33 ^a	58.25±10.52 ^a	28.40±2.99 ^a	41.82±3.93 ^a
	PG	31.31±0.25 ^b	46.14±7.41 ^b	10.23±2.09 ^b	23.98±0.52 ^b
	IN	5.94±1.28 ^c	23.06±3.06 ^c	5.25±1.11 ^c	5,24±1.00 ^d
	OUT	7.54±0.42 ^c	25.28±4.31 ^c	5.62±1.99 ^c	14.97±0.71 ^c
	RECOVERY %	10.6	39.6	18.5	12.5

*: mg TEAC/100 mL

Cilla et al. (2011), studied the influence of storage and *in vitro* gastrointestinal digestion on total antioxidant capacity of fruit beverages. Samples were manufactured with the same raw materials in order to minimize variability. Eight fruit beverages were used [all of them made of white grape concentrate of the “Aire’n” variety (initial 68° Brix diluted to a final ~11° Brix), sweet orange concentrate of the “Hamlin” variety (initial 65 brix diluted to a final 11.2° Brix), apricot puree of the “Bullida” variety (11.5° Brix), and peach puree of the “Carson” variety (12° Brix)], with/without iron (Fe) and/or zinc (Zn) and with/without skimmed milk. Total antioxidant capacity obtained in freshly manufactured fruit beverages was reported as 5826±202 µM TEAC/L sample for ABTS assay and 1181±17 mg of GAE/L sample for total phenols assay. Bioaccessibility of fruit beverages changed between 6517±172 µM TEAC/L sample to 1066±33 mg of GAE/L sample for same analysis.

The phenolic contents of Cabernet Franc, Merlot, Sangiovese, Syrahred wines known as Brazilian red wines, from the 2007 and 2006 vintages were found between 2287.6±31.1 and 2790.5±34.2 mg GAE/L (Gris et al., 2013). In addition to this plasma antioxidant capacity was measured with FRAP analysis and increases in the plasma antioxidant activity ranged from 17.8% to 70.7% when compared to the control groups.

Besides measuring TPC, Modun et al., (2008) evaluated the total antioxidant activity of red wine (RW) and dealcoholized red wine (DARW) samples with FRAP method and results were 11.472±0.212 and 11.495±0.250 mmol/L TE for DARW and RW, respectively. FRAP values after consumption were found to be 3781±684 and 6127±687 µmol/L for DARW and RW, respectively. These values indicate 32.9% and 53.3% recovery rates. It was concluded

that TAC of grape vinegar is lower than DARW and RW. However they also stood on a point that *in vitro* FRAP values of samples had not followed to the changes in FRAP plasma values obtained after consumption of these beverages, and they explained this with the elevation in plasma urate, a phenomenon that occurs only in *in vivo* conditions.

4.4.2 The Effects of *In Vitro* Bioaccessibility on Apple Vinegar

Apple vinegar, supplied from Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions were included analysis. Besides this, IN fraction was checked against initial value of apple vinegar and this comparison amount was reported in percent at "Recovery" section, too.

4.4.2.1 Changes in Total Phenolic and Total Flavonoid Contents

TFC and TPC of the PG, IN and OUT fractions of apple vinegar are given in Table 4.12 on FWB. Results are given in mg CA/100 mL samples for TFC and mg GAE/100 mL samples for TPC, significant difference was determined between the samples ($p < 0.05$). High total flavonoid recovery values might be related with the standard deviation of the analytical method used. However, still taking this information into account, it can be concluded that high recovery values were obtained for total flavonoid content of apple vinegar sample. On the other hand the recovery rate for phenolic content is more reasonable for apple vinegar, which was found to be 73.8%.

Table 4.12: Total flavonoid and phenolic contents of apple vinegar and *in vitro* digested apple vinegar samples.

Apple Vinegar		TFC*	TPC**
	INITIAL	2.36±0.99 ^b	16.86±1.12 ^a
	PG	3.70±0.23 ^a	12.87±2.43 ^b
	IN	2.38±0.16 ^b	12.45±1.86 ^b
	OUT	2.94±0.34 ^b	15.83±3.73 ^{ab}
	RECOVERY %	100.8	73.8

* : mg CA/100 mL samples

** : mg GAE/100 mL samples

Bouyed et al. (2011), studied on simulated gastro-intestinal digestion and dialysis on apple varieties which were Jonaprinz, Jonagold, Golden and Mutzu, the first two varieties being of red, the others of green skin. It was reported that there was augmentation from gastric stage to

intestinal digesta for all apple varieties, although considering the recoveries of apples while comparing dialysable phenolics of them, there was a decline at recovery rate between 44.6% and 62.7% values. Furthermore, flavonoid content of apples were detected after gastric and intestinal phase however dialysable flavonoids were found significantly inferior, and recovery of apple varieties changed approximately from 26.3% to 51.8%, respectively. The outcomes of our results showed elevated amounts of TFC and TPC. In general fruits had higher TPC values. Recovery percentage of samples may not reflect the actual situation as the apple vinegar had very low TFC and TPC values initially.

4.4.2.2 Changes in Antioxidant Capacity

Antioxidant capacity of the Total PG, IN and OUT fractions of apple vinegar are shown in Table 4.13 on FWB. Fractions were evaluated with ABTS, CUPRAC, DPPH and, FRAP assays. Results are given in mg TEAC/100 mL samples for all antioxidant capacity assays, significant difference was determined between the samples ($p < 0.05$). Initial quantities of apple vinegar were found 36.64 ± 7.12 , 48.08 ± 8.20 , 13.31 ± 1.26 and 29.57 ± 4.02 mg TEAC/100 mL with the sequence of ABTS, CUPRAC, DPPH and FRAP, IN fraction TAC values were 7.93 ± 2.06 , 20.99 ± 2.45 , 3.06 ± 1.20 and 3.33 ± 0.68 mg TEAC/100 mL with the same sequence of ABTS, CUPRAC, DPPH and FRAP.

Table 4. 13: Antioxidant capacity of apple vinegar and *in vitro* digested apple vinegar samples.

Apple Vinegar		ABTS*	CUPRAC*	DPPH*	FRAP*
	INITIAL	36.64 ± 7.12^a	48.08 ± 8.20^a	13.31 ± 1.26^a	29.57 ± 4.02^a
	PG	24.49 ± 0.59^b	29.40 ± 3.02^b	6.68 ± 3.72^b	13.25 ± 1.51^b
	IN	7.93 ± 2.06^c	20.99 ± 2.45^c	3.06 ± 1.20^c	3.33 ± 0.68^c
	OUT	5.99 ± 0.45^c	14.57 ± 4.96^c	4.51 ± 1.88^{bc}	5.17 ± 1.18^c
	RECOVERY %	21.6	43.6	23.0	11.3

*: mg TEAC/100 mL

Lotito and Frei (2004b), studied the increase in human plasma antioxidant capacity after apple consumption. Six healthy subjects were chosen and plasma was obtained from them before and up to 6 h after consumption of five apples. After apple consumption researchers determined that FRAP results increased after 1 hour by 12%. The observed variety between *in*

vitro and *in vivo* results after apple intake can be attributed to the enhancement of uric acid in serum and its effect on the antioxidant capacity, whereas polyphenols and ascorbic acid play a negligible role.

Vieira et al. (2012), investigated the improvement of serum antioxidant status in humans after the acute intake of apple juices. They confirmed with this study, acute consumption of juices from 2 apple cultivars, Golden Delicious and Catarina juices, with different sugars, ascorbic acid, and phenols content and antioxidant capacities promoted a similar effect on the serum antioxidant status and lipid peroxidation of healthy subjects. 1 hour after the consumption of either Golden Delicious and Catarina apple juice an increase in the antioxidant capacity of serum was observed, measured by ABTS and FRAP analyses, sequentially 4.2% and 10.2%, 5.5% and 10.6%, in relation to the baseline values or in comparison with the water intake. Researchers associated the increase of the serum antioxidant capacity with uric acid levels after the intake of Golden Delicious ($r = 0.725$) and Catarina ($r = 0.903$) and they added, although the high *in vitro* antioxidant capacity of fruits, their intake does not appear to result in correspondent *in vivo* antioxidant effects in plasma.

It is cleared with both of these researches, albeit apple vinegar's high antioxidant recovery rate, *in vitro* GI may not mirror the same results with *in vivo* digestion. Lotito and Frei (2004b), added apple extracts to the human plasma *in vitro* and compared the result with the *in vivo* assay. They observed that total phenols showed significant effects in plasma *in vitro*, however The lack of effect in the *in vivo* study may have several reasons, including very low or negligible absorption of procyanidins and metabolic conversion of the absorbed apple polyphenols.

4.4.3 The Effects of *In Vitro* Bioaccessibility on Pomegranate Vinegar

Pomenranate vinegar, supplied by Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions are analyzed. As with the other samples, IN fraction was checked against initial value of pomegranate vinegar and this comparison amount reported in percent at "Recovery" section.

4.4.3.1 Changes in Total Phenolic and Total Flavonoid Contents

TFC and TPC of the PG, IN and OUT fractions of pomegranate vinegar are displayed together at Table 4.14 on FWB. Results are given in mg CA/100 mL samples for TFC and mg

GAE/100 mL samples for TPC, significant difference was determined between the samples ($p<0.05$). Recovery percentage of phenolic contents are higher than that of flavonoid contents.

Table 4.14: Total flavonoid and phenolic contents of pomegranate vinegar and *in vitro* digested pomegranate vinegar samples.

Pomegranate Vinegar		TFC*	TPC**
	INITIAL	15.51±3.65 ^a	89.49±4.48 ^a
	PG	10.14±0.48 ^b	57.96±2.68 ^b
	IN	2.78±0.45 ^d	18.20±1.95 ^d
	OUT	6.58±0.33 ^c	33.73±5.62 ^c
	RECOVERY %	17.9	20.3

* : mg CA/100 mL samples

** : mg GAE/100 mL samples

Caliskan and Bayazit (2012), investigated the diversity in phytochemical characteristics among the 76 pomegranate accessions grown in the eastern Mediterranean region of Turkey. They reported that all pomegranates from Turkey had total phenolic capacity range of 108.0-944.9 mg GAE/100 g juice which are considerable as high level of phenolic content. Comparison of these juice values with vinegar samples initial values indicates that fermentation step may have lowered TPC values of pomegranate juice. On the other hand, recovery percentage of pomegranate vinegar is acceptable for TFC and TPC.

4.4.3.2 Changes in Antioxidant Capacity

TAC of the PG, IN and OUT fractions of pomegranate vinegar are given in Table 4.15 on FWB. Fractions were measured with ABTS, CUPRAC, DPPH and, FRAP analyses. Results are given in mg TEAC/100 mL samples for all antioxidant capacity assays, additionally significant difference was determined between the samples ($p<0.05$). Initial amounts of antioxidants in pomegranate vinegar were found to be much higher than IN fraction. This situation caused nominal rate on recovery percentage.

Table 4.15: Antioxidant capacity of pomegranate vinegar and *in vitro* digested pomegranate vinegar samples.

Pomegranate Vinegar		ABTS*	CUPRAC*	DPPH*	FRAP*
	INITIAL	189.10±21.35 ^a	260.95±52.53 ^a	153.58±20.66 ^a	171.23±18.59 ^a
	PG	34.24±0.68 ^b	162.64±6.53 ^b	107.68±3.00 ^b	30.35±4.26 ^b
	IN	3.43±0.87 ^c	29.86±2.38 ^c	0.54±0.25 ^c	9.18±1.63 ^d
	OUT	16.33±0.76 ^c	68.33±4.61 ^c	12.75±1.98 ^c	24.07±3.08 ^c
	RECOVERY %	1.8	11.4	0.3	5.4

*: mg TEAC/100 mL

Previously mentioned study of Gil et al. (2000) on process effect on antioxidant activity of grape vinegar section, Cabernet Sauvignon red wine was used to evaluate values of experimental pomegranate juice obtained from fresh arils and experimental juice prepared from frozen arils by utilizing the ABTS and the DPPH methods and they reported that the antioxidant activity of the experimental pomegranate juice obtained from fresh arils was twice those of red wine but lower in the experimental juice prepared from frozen arils. This occasion indicating that during the freezing process some antioxidant compounds are degraded or transformed, but the juice still showed a higher antioxidant activity than red wine. The FRAP method was also applied to the samples and similarly, the activity of the commercial juices was higher than red wine.

Çam et al. (2009), studied on classification of eight pomegranate juices based on antioxidant capacity and according to results they found that pomegranate juice's antioxidant capacity changed among 221.2±2.4 and 418.3±5.2 mg TEAC/100 mL for ABTS assay.

4.4.4 The Effects of *In Vitro* Bioaccessibility on Balsamic Vinegar

Balsamic vinegar, provided from Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions are analyzed. Furthermore, IN fraction was checked against initial value of balsamic vinegar and this comparison amount reported in percent at "Recovery" section.

4.4.4.1 Changes in Total Phenolic and Total Flavonoid Contents

TFC and TPC of the PG, IN and OUT fractions of pomegranate vinegar are presented in Table 4.16 on FWB. Results are given in mg CA/100 mL samples for TFC and mg GAE/100 mL samples for TPC, significant difference was determined between the samples ($p < 0.05$). Balsamic vinegar with values of 96.13 ± 18.31 for TFL and 254.66 ± 24.38 for TFE had the highest initial values between all samples. Nevertheless its recovery percentage is quite less than other vinegars which were analyzed for *in vitro* bioaccessibility, however its recovery amounts are more than them anyway.

Table 4.16: Total flavonoid and phenolic contents of balsamic vinegar and *in vitro* digested balsamic vinegar samples.

Balsamic Vinegar		TFC*	TPC**
	INITIAL	96.13 ± 18.31^a	254.66 ± 24.38^a
	PG	53.70 ± 1.28^b	225.99 ± 17.21^b
	IN	3.05 ± 0.44^d	34.12 ± 2.87^d
	OUT	37.26 ± 3.22^c	166.98 ± 5.71^c
	RECOVERY %	3.2	13.1

* : mg CA/100 mL samples

**:.mg GAE/100 mL samples

Verzelloni et al. (2007), evaluated the relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsamic vinegar. Results from this work were 58.06 mg CA/100 ml for traditional balsamic vinegar, 33.47 mg CA/100 ml for balsamic vinegar in the methanolic fractions for phenolic content and the flavonoid content in the aqueous fractions was zero for all of the samples.

Masino et al. (2008), studied the relationships among chemical, physical, and qualitative assessment in traditional balsamic vinegar. They investigated nineteen commercially available samples of traditional balsamic vinegar, the total phenolic content of samples changed 1460 mg GAE/kg sample to 5430 mg GAE/kg sample.

Kamiloglu & Capanoglu (2013), studied on *in vitro* gastrointestinal digestion of polyphenols from different molasses and leather varieties. The results indicated that initial values of grape molasses and grape leather were 14 ± 2 and 16 ± 3 , PG values were 18 ± 1 and 15 ± 2 , IN values were 2.5 ± 0.4 and 3.6 ± 0.5 , and also OUT values were 16 ± 3 and 19 ± 2 mg CA/100 g dry

weight for flavonois content. Besides this recovery rate range to 17-9% for grape molasses and grape leather. In the case of evaluating the total phenolic content of grape molasses and grape leather they found 125 ± 5 and 72 ± 8 for initial values, 109 ± 6 and 189 ± 9 for PG values, 26 ± 5 and 36 ± 4 for IN values, 91 ± 4 and 179 ± 7 mg GAE/100 g dry weight for OUT values at phenolic content analyze and recovery rate varied to 21-50% for grape molasses and grape leather.

The analyzed balsamic vinegar in our study had a similar place between the nineteen commercially available samples of traditional balsamic vinegar studied by Masino et al. (2008). In addition to these balsamic vinegar had higher TFC and TPC values than grape molasses and grape leather. Even so, recovery rateS of balsamic vinegar are lesser than either grape molasses and grape leather for both analysis.

4.4.4.2 Changes in Antioxidants Capacity

Antioxidant capacity of the Total PG, IN and OUT fractions of balsamic vinegar are given in Table 4.17 on FWB. Fractions were measured with ABTS, CUPRAC, DPPH and, FRAP analyses. Results are given in mg TEAC/100 mL samples for all antioxidant capacity essay, additionally significant difference was determined between the samples ($p<0.05$). Recovery percentage of balsamic vinegar in antioxidant capacity essays are low again when considering its initial amounts had the highest values in all samples. This conditions could not affect the having utmost amount at IN fraction's results in antioxidant capacity essay too.

Table 4.17: Antioxidant capacity balsamic vinegar and *in vitro* digested balsamic vinegar samples.

Balsamic Vinegar		ABTS*	CUPRAC*	DPPH*	FRAP*
	INITIAL	250.05 ± 42.12^a	738.08 ± 79.80^a	281.09 ± 42.91^a	420.84 ± 28.37^a
	PG	270.70 ± 6.11^a	444.18 ± 40.99^b	165.29 ± 9.01^b	275.65 ± 13.18^b
	IN	13.07 ± 0.54^b	48.19 ± 9.60^d	6.46 ± 2.79^c	11.24 ± 1.58^d
	OUT	25.53 ± 7.23^b	259.41 ± 47.62^c	21.13 ± 8.26^c	101.40 ± 14.71^c
	RECOVERY %	5.2	6.5	2.3	2.7

*: mg TEAC/100 mL

In a study by Masino et al. (2008) radical scavenging analysis were performed with ABTS assay for traditional balsamic vinegar. ABTS values were reported to range from 14.5 mM to 58.2 mM TEAC.

Tagliazucchi et al. (2008) investigated the antioxidant properties of traditional balsamic vinegar in relation to its content in polyphenols, polymeric tannins and Maillard Reaction products. According to their opinion Maillard Reaction has an effect on development of characteristic aroma, taste and color of balsamic vinegar so it is reasonable to think that during the cooking of must, Maillard Reaction products with antioxidant activity are synthesized and could be responsible for the high antioxidant activity of traditional balsamic vinegar. Maillard Reaction may occur in boiled grape juice because of its high content of reducing sugars and amino groups (Göğüş et al., 1998).

The study of Tagliazucchi et al., (2008) pointed that traditional balsamic vinegar has a high antioxidant activity due to polyphenols and tannins and Maillard Reaction products and they also denoted that independent from their bioaccessibility, using traditional balsamic vinegar as a sauce can contribute, along with vegetables and wine, to an increase in the total amount of antioxidants ingested during a meal.

In the continuation of research of Kamiloglu & Capanoglu (2013), authors found the recovery percentage of grape molasses were 35% for ABTS, 18% for DPPH, 17% for CUPRAC, 24% for FRAP assays and grape leather were 38%, 3%, 36%, 25% with the same analyze alignment.

TAC values of grape molasses and grape leather were still higher than balsamic vinegar in TFC and TPC tests considering the recovery rate. In general, balsamic vinegar had the highest initial values in TFC, TPC and TAC assays, however their recovery percentages were found to be very low considering initial values. Nevertheless, the amount of TFC, TPC and TAC at IN fraction, still had a high quantity.

4.5 The Effects of *In Vitro* Bioaccessibility on Vinegar Phenolic Profile

The *in vitro* bioaccessibility of vinegar samples were also investigated in this study as mentioned because of the lack of information about vinegar digestion in the literature. The

samples which were directly applied in vitro bioaccessibility method took into HPLC-PDA analysis for PG, IN and OUT fraction of vinegars.

4.5.1 The Effects of *In Vitro* Bioaccessibility on Grape Vinegar

Grape vinegar, supplied by Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions were analyzed. Furthermore, IN fraction was checked against initial value of grape vinegar and this comparison amount was reported in percent at "Recovery" section. Phenolic compounds of the samples were measured with HPLC-PDA and gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.18 and results of samples were given as mg /100 mL samples.

Table 4.18: The effects of *in vitro* bioaccessibility procedure on grape vinegar phenolic compounds.

Grape Vinegar		Gallic Acid	<i>p</i>-Hydroxybenzoic Acid
	INITIAL	5.16±0.08	0.45±0.00
	PG	1.63±0.01	0.14±0.07
	IN	0.66±0.01	0.03±0.01
	OUT	0.77±0.04	0.01±0.00
	RECOVERY %	12.8	6.7

*Results were given in mg Standart/100 mL samples for all.

**N.D. : Not dedected.

Results indicated that initial values of phenolic acids in generally disappeared after *in vitro* digestion. The highest recovery percentage were observed at gallic acid concentration with 12.8%.

4.5.2 The Effects of *In Vitro* Bioaccessibility on Apple Vinegar

Grape vinegar, supplied by Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions were analyzed. Furthermore, IN fraction was checked against initial value of grape vinegar and this comparison amount was reported in percent at "Recovery" section. Phenolic compounds of the samples were measured with HPLC-PDA and

gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.19 and results of samples were given as mg /100 mL samples.

Table 4.19: The effects of *in vitro* bioaccessibility procedure on apple vinegar phenolic compounds.

Apple Vinegar		Gallic Acid	<i>p</i> -Hydroxybenzoic Acid	Catechin
	INITIAL	0.92±0.05	0.34±0.05	2.27±0.01
	PG	0.31±0.01	0.09±0.00	0.09±0.01
	IN	0.18±0.01	0.03±0.01	0.38±0.07
	OUT	0.21±0.01	0.02±0.00	0.66±0.02
	RECOVERY %	19.6	8.8	1.7

*Results were given in mg /100 mL samples for all.

**N.D. : Not detected.

Predetermined phenolics were found in initial stage of apple vinegar with little amounts. Despite this *invitro* digestion procedure affected their bioaccessibility and their concentration was found at very low levels. Recovery percentage of gallic acid is the highest one as happened at grape vinegar recovery with 19.6%.

4.5.3 The Effects of *In Vitro* Bioaccessibility on Pomegranate Vinegar

Grape vinegar, supplied by Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions were analyzed. Furthermore, IN fraction was checked against initial value of grape vinegar and this comparison amount was reported in percent at "Recovery" section. Phenolic compounds of the samples were measured with HPLC-PDA and gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.20 and results of samples were given as mg /100 mL samples.

Table 4.20: The effects of *in vitro* bioaccessibility procedure on pomegranate vinegar phenolic compounds.

Pomegranate Vinegar		Gallic Acid	Protocatechuic Acid	<i>p</i> -Hydroxybenzoic Acid	Catechin	Syringic Acid
	INITIAL	7.94±0.18	3.51±0.03	6.05±0.23	12.57±1.18	0.41±0.03
	PG	1.52±0.05	0.24±0.02	1.58±0.06	3.90±0.16	0.13±0.01
	IN	0.50±0.02	0.08±0.00	0.02±0.01	0.27±0.03	0.01±0.00
	OUT	0.55±0.02	0.08±0.01	0.06±0.00	0.47±0.04	0.04±0.00
	RECOVERY %	6.3	2.3	0.3	2.1	2.4

*Results were given in mg /100 mL samples for all.

Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, catechin, syringic acid were found at initial level of pomegranate vinegar. Despite their initial amount of phenolic compounds, their recovery percentages were found nominal.

4.5.4 The Effects of *In Vitro* Bioaccessibility on Balsamic Vinegar

Grape vinegar, supplied by Kuhne Vinegar Factory, was used for *in vitro* bioaccessibility analysis. PG, IN and OUT fractions were analyzed. Furthermore, IN fraction was checked against initial value of grape vinegar and this comparison amount was reported in percent at "Recovery" section. Phenolic compounds of the samples were measured with HPLC-PDA and gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.21 and results of samples were given as mg /100 mL samples.

Table 4.21: The effects of *in vitro* bioaccessibility procedure on balsamic vinegar phenolic compounds.

Balsamic Vinegar		Gallic Acid	Caffeic Acid	<i>p</i>-Coumaric Acid
	INITIAL	83.02±0.67	0.40±0.13	0.20±0.04
	PG	21.38±0.24	0.06±0.01	0.05±0.00
	IN	9.10±0.00	0.03±0.00	0.01±0.00
	OUT	7.29±0.00	0.03±0.00	0.01±0.00
	RECOVERY %	11.0	7.5	5.0

*Results were given in mg /100 mL samples for all.

Gallic acid, caffeic acid and *p*-coumaric acid were found at balsamic vinegar and *in vitro* fractions of balsamic vinegar considering predetermined phenolic compounds. Initial level of them were 83.02±0.67, 0.40±0.13 and 0.20±0.04 mg /100 mL sample, respectively. The most elevated recovery percentage observed at gallic acid 11.0% and caffeic acid with 7.5% and this was chased by *p*-coumaric acid with 5.0%.

4.6 Comparasion of Antioxidant Content of Different Vinegar Types

In this section of the study every single one of the vinegar samples, provided from different factories, produced with different manufacturing process and ready to use final products, were compared with each other with respect to their total flavonoid content, total phenolic content and antioxidant capacity assays. Vinegar samples were enumerated while investigating the antioxidant capacity and phenolic-flavonoid content of different vinegar samples. Apple vinegar I, Grape vinegar I and Pomegranate Vinegar I belong to Kuhne Vinegar Factory, Pomegranate Vinegar II belongs to Yedier Vinegar Factory, and Grape Vinegar II, Apple Vinegar II belong to Nahita Natural Fermentation Factory.

4.6.1.1 Changes in Total Phenolic and Total Flavonoid Contents

Total flavonoid content and total phenolic content of the different types of vinegar are presented in Table 4.22 and Figure 4.1 on FWB. Results are given in mg CA/100 mL samples for total flavonoid content and mg GAE/100 mL samples for total phenolic content,

significant difference was determined between the samples ($p < 0.05$). Among vinegar samples analyzed for flavonoid and phenolic content, balsamic vinegar had the greatest value with 96.13 ± 18.31 for TFL and 254.66 ± 24.38 for TFE. Results indicated that rosehip vinegar and gilaburu vinegar were following balsamic vinegar with the amounts of 34.19 ± 5.62 and 31.93 ± 10.81 , respectively, at flavonoid content while blueberry vinegar was coming after with the amount of 93.04 ± 12.17 for phenolic content. Conversely apple vinegar I had the lowest TFL ve TFE results with consecutively 2.36 ± 0.99 and 16.86 ± 1.12 values.

Table 4.22: Total flavonoid and phenolic contents of some vinegars.

	TFC*	TPC**
Apple Vinegar I	2.36 ± 0.99^e	16.86 ± 1.12^h
Grape Vinegar I	5.52 ± 0.97^{cde}	26.38 ± 1.29^{gh}
Pomegranate Vinegar I	15.51 ± 3.65^c	89.49 ± 4.48^{bc}
Balsamic Vinegar	96.13 ± 18.31^a	254.66 ± 24.38^a
Blueberry Vinegar	7.52 ± 0.31^{cde}	93.04 ± 12.17^b
Rosehip Vinegar	34.19 ± 5.62^b	72.33 ± 17.83^{cd}
Pomegranate Vinegar II	4.87 ± 0.24^{de}	41.69 ± 2.44^{fg}
Grape Vinegar II	11.79 ± 0.32^{cde}	67.52 ± 3.30^{de}
Gilaburu Vinegar	31.93 ± 10.81^b	80.78 ± 20.32^{bcd}
Apple Vinegar II	5.42 ± 0.15^{de}	31.01 ± 4.39^{fgh}
Lemon Vinegar	6.09 ± 1.25^{cde}	27.47 ± 8.56^{gh}
Blackberry Vinegar	9.45 ± 1.68^{cde}	48.59 ± 7.32^{ef}
Artichoke Vinegar	13.26 ± 3.30^{cd}	44.34 ± 17.63^{fg}
Mulberry Vinegar	7.69 ± 0.22^{cde}	29.78 ± 1.21^{fgh}
Rice Vinegar	5.92 ± 0.47^{cde}	24.24 ± 0.76^{gh}
Apricot Vinegar	5.58 ± 0.20^{cde}	25.93 ± 1.44^{gh}
Date Vinegar	7.76 ± 0.16^{cde}	43.17 ± 4.38^{fg}
Howthorn Vinegar	5.23 ± 1.75^{de}	28.22 ± 0.90^{gh}

* : mg CA/100 mL samples

**:.mg GAE/100 mL samples

***Apple vinegar I, Grape vinegar I and Pomegranate Vinegar I belong to Kuhne Vinegar Factory, Pomegranate Vinegar II belongs to Yedier Vinegar Factory, and Grape Vinegar II, Apple Vinegar II belong to Nahita Natural Fermentation Factory.

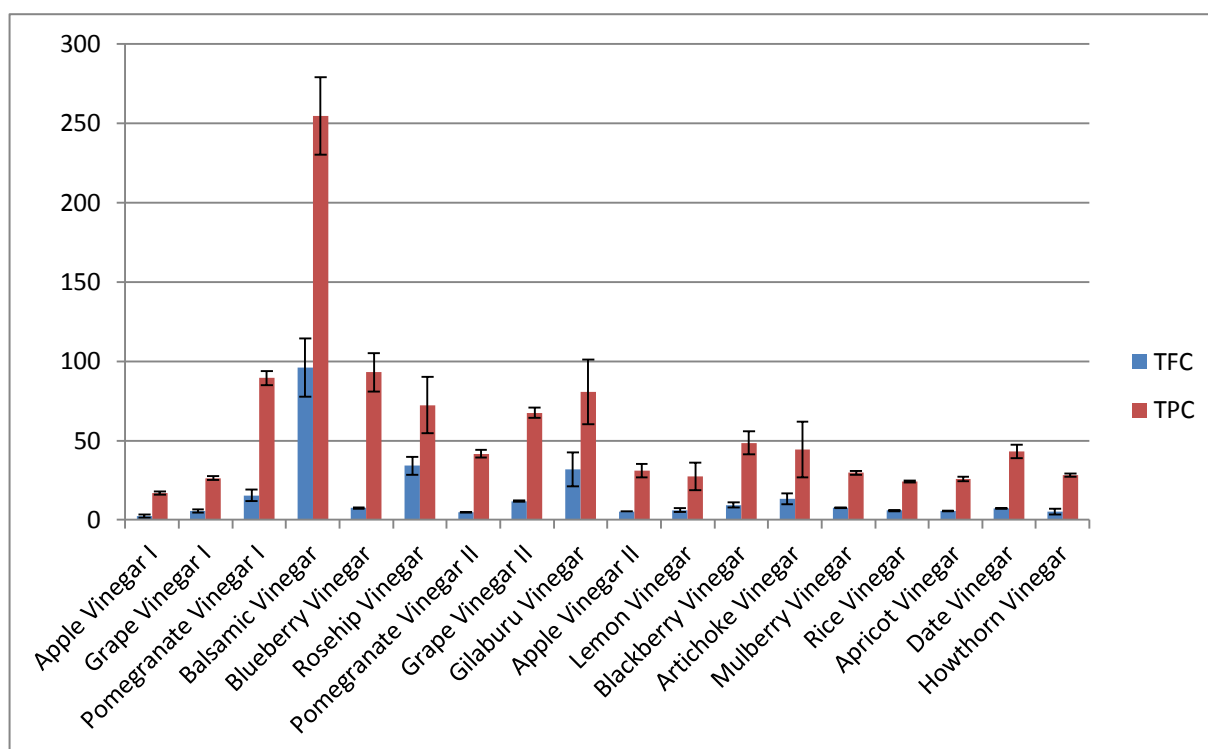


Figure 4.1: Total flavonoid, phenolic contents of some vinegars.

*Apple vinegar I, Grape vinegar I and Pomegranate Vinegar I belong to Kuhne Vinegar Factory, Pomegranate Vinegar II belongs to Yedier Vinegar Factory, and Grape Vinegar II, Apple Vinegar II belong to Nahita Natural Fermentation Factory.

Diversed type of vinegars are consumed all around the world with different people for different purposes. Consuming vinegar products for their nutritious properties is an emerging trend in Asia. Rice vinegars have been a part of local culture and extremely used in the cuisines for many years. A drinking vinegar, *Moromi* vinegar, one of the most prominent health vinegars in the Asia Pacific region and originated from southern Japan, blended with onion vinegar and brown sugar juice for flavour, is rich in nutrients and high in amino acids. In addition to these, Japan has also recently experienced a trend in colourful foods such as black vinegar, especially two black vinegar drinks, mixed with honey lemon, blueberries, and additional supplements for health conscious consumers were launched in the market. People of Latin America interest in herb-infused and fruit flavoured vinegars such as apples, berries, herbs, honey may prove most popular amongst consumers. Brazilians consume balsamic, red, and white wine vinegars according the sales rates of markets. It is seen to be taking into account the Middle East, blueberry and date vinegar is more popular in here. Besides these

specific fruit varieties that may be well received are apple, cranberry, raspberry, honey, and potato (Berry, 2011).

A study made by Cerezo et al., (2008) evaluated the phenolic composition of red wine vinegar produced in barrels made of different woods. For this purpose they monitored the phenolics during the acetification of red wine vinegars produced by surface culture in different wood barrels made of oak, chestnut, acacia and cherry. They found submerged culture acetification of red wine vinegars resulted in a 13% reduction in polyphenols.

Another study implemented by Cerezo et al. (2010), investigated the effect of wood on the phenolic profile and sensory properties of wine vinegars and balsamic vinegar during ageing. It was reported that wood type had an effect on total phenolic content. Utilizing cherry, chestnut or oak woods for aging increased the phenolic content in balsamic vinegar about 30-50%. According the results samples aged in acacia wood did not show significant changes in the phenolic contents during ageing but, samples aged in chestnut wood increased significantly.

Lo'pez et al.,(2005) studied the clarification of vinegars (white, rose and red) by cross-flow microfiltration on an industrial scale. It was indicated that using microfiltration affected colour and polyphenol content but it was in an acceptable range. The reduction in polyphenol content was less than 15% in all vinegars, and was higher for red vinegar, followed by rose and finally white vinegar. Despite the polyphenolic profile of the filtered vinegars was quite similar to that of the initial vinegar, the decrease in the polyphenol content was related to the reduction in colour and they concluded that while the process had no influence on water soluble phenolic compounds, flavonoid compound, that could be identified in the samples analysed was procyanidin B2, was found in vinegars before filtration but it could not be detected in filtered samples.

In our study, vinegars, produced with different production methods and dissimilar content of fruits vegetables, herb or legume had varied effects on TFC,TPC and TAC content. This occasion can be observed easily at same vinegar kind that produced by different companies with distinct methods. Apple, grape and pomegranate vinegars were evaluated for this purpose with each others derivative obtained from different production methods . Apple vinegar II doubled itself TFC value rather than apple vinegar I, while grape vinegar II passed

twice of grape vinegar I TFC value. Alike, TPC amount of apple vinegar I has half of apple vinegar II, grape vinegar II has 2.55 times of TPC rather than grape vinegar I. However this situation is not suitable for pomegranate vinegar kinds. In contrast to apple and grape vinegar, pomegranate vinegar I had more TFC and TPC than pomegranate vinegar II, more than 3 times and more than 2 times, respectively.

4.6.2 Changes in Antioxidant Capacity

Antioxidant capacity of different types of vinegar are given in Table 4.23 and Figure 4.2 on FWB. Vinegars were tested using ABTS, CUPRAC, DPPH and, FRAP assays. Results are given in mg TEAC/100 mL samples for all antioxidant capacity assays, additionally significant difference was determined between the samples ($p < 0.05$). ABTS and DPPH results demonstrated that rosehip vinegar had the highest antioxidant capacity with 253.18 ± 44.69 and 517.05 ± 43.40 mg TEAC/100 mL values followed by blueberry vinegar, balsamic vinegar and gilaburu vinegar. Considering the CUPRAC and FRAP results, balsamic vinegar had the highest antioxidant capacity followed by rosehip vinegar. Comparing the apple vinegar I and apple vinegar II indicated that there was no significant difference between these samples; however crosscheck among grape vinegar I and grape vinegar II as well as pomegranate vinegar I and pomegranate vinegar II indicated that there is a significant difference between those.

Table 4.23: Antioxidant capacity of some vinegars.

	ABTS*	CUPRAC*	DPPH*	FRAP*
Apple Vinegar I	36.64±7.12 ^g	48.08±8.20 ^g	13.31±1.26 ⁱ	29.57±4.02 ^f
Grape Vinegar I	55.88±6.33 ^{fg}	58.25±10.2 ^g	28.40±2.99 ^{hi}	41.82±3.93 ^{ef}
Pomegranate Vinegar I	189.10±21.35 ^{cd}	260.95±52.53 ^{bc}	153.58±20.66 ^e	171.23±18.59 ^c
Balsamic Vinegar	250.05±42.12 ^{bc}	708.67±107.83 ^a	281.09±42.91 ^d	420.84±28.37 ^a
Blueberry Vinegar	373.32±19.01 ^a	263.96±80.82 ^{bcd}	448.32±10.59 ^b	135.75±8.74 ^c
Rosehip Vinegar	253.18±44.69 ^b	327.43±52.39 ^b	517.05±43.40 ^a	210.29±52.44 ^b
Pomegranate Vinegar II	40.35±0.28 ^{fg}	67.70±4.14 ^g	16.35±0.74 ⁱ	18.97±0.24 ^f
Grape Vinegar II	100.37±2.33 ^{de}	169.65±20.56 ^{ef}	132.37±2.02 ^{ef}	82.84±3.89 ^d
Gilaburu Vinegar	90.65±2.22 ^{bcd}	223.86±63.14 ^{cde}	329.69±47.88 ^c	151.70±45.48 ^c
Apple Vinegar II	32.56±0.29 ^{fg}	57.32±6.66 ^g	21.07±1.54 ⁱ	24.86±1.40 ^f
Lemon Vinegar	38.86±3.68 ^{fg}	72.12±6.84 ^g	44.11±9.74 ^{hi}	41.15±7.21 ^{ef}
Blackberry Vinegar	45.92±0.05 ^{ef}	117.39±8.86 ^{fg}	69.47±0.40 ^{gh}	82.14±3.49 ^d
Artichoke Vinegar	79.19±29.32 ^{cd}	191.27±27.41 ^{def}	100.51±34.54 ^{fg}	73.99±28.69 ^{de}
Mulberry Vinegar	40.83±0.73 ^{fg}	76.34±4.67 ^g	46.18±9.97 ^{hi}	42.80±1.32 ^{ef}
Rice Vinegar	38.67±0.76 ^{fg}	67.65±4.03 ^g	24.82±1.33 ⁱ	32.00±1.11 ^f
Apricot Vinegar	37.03±0.65 ^{fg}	58.38±2.86 ^g	33.60±1.31 ^{hi}	30.09±1.43 ^f
Date Vinegar	45.95±0.06 ^{fg}	83.41±6.23 ^{gh}	48.42±0.94 ⁱ	50.46±2.36 ^{def}
Howthorn Vinegar	38.04±0.63 ^{fg}	63.33±2.43 ^g	40.82±0.74 ^{hi}	45.37±1.42 ^{def}

*: mg TEAC/100 mL

Apple vinegar I, Grape vinegar I and Pomegranate Vinegar I belong to Kuhne Vinegar Factory, Pomegranate Vinegar II belongs to Yedier Vinegar Factory, and Grape Vinegar II, Apple Vinegar II belong to Nahita Natural Fermentation Factory.

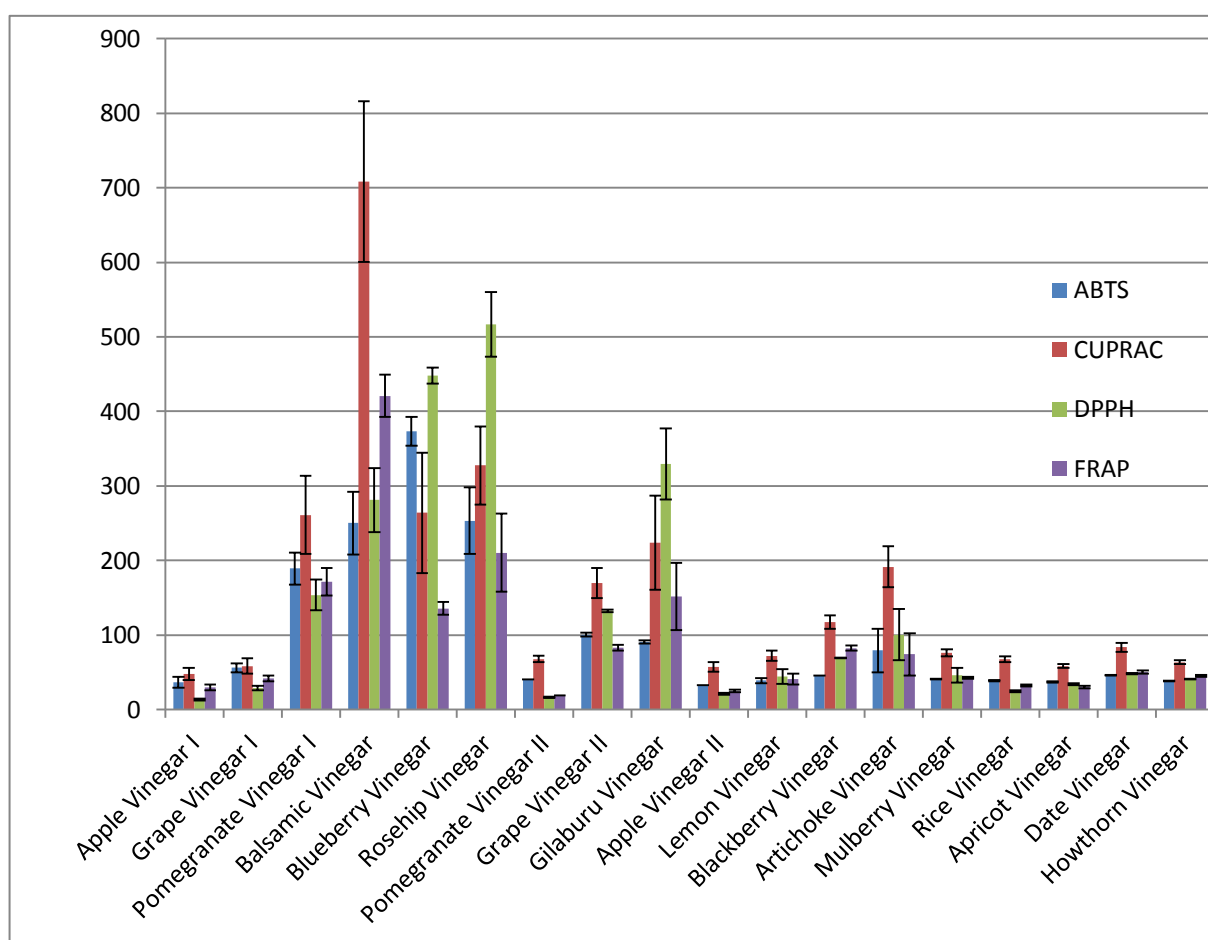


Figure 4.2: Antioxidant capacity of some vinegars.

Apple vinegar I, Grape vinegar I and Pomegranate Vinegar I belong to Kuhne Vinegar Factory, Pomegranate Vinegar II belongs to Yedier Vinegar Factory, and Grape Vinegar II, Apple Vinegar II belong to Nahita Natural Fermentation Factory.

Similar to TFC and TPC results, apple vinegar I and grape vinegar I had less antioxidant content (TAC) than apple vinegar II and grape vinegar II, whereas pomegranate vinegar I had more than pomegranate vinegar II with all TAC assays.

Addition of sodium metabisulfite as an antioxidant in pomegranate vinegar I and utilizing mollasses as a supplement of vinegar production in pomegranate vinegar II could be leading to this result. Another possible reason might be the different dry matter contents in samples (13.5° Brix for pomegranate vinegar I and 2.3° Brix for pomegranate vinegar II).

4.7 The Comparasion of Phenolic Compounds of Different Vinegar Types

In this section of the study every single one of the vinegar samples, provided from different factories, produced with different manufacturing process and ready to use final products, were compared with each other with respect to their phenolic content. Vinegar samples were enumerated while investigating the antioxidant capacity and phenolic-flavonoid content of different vinegar samples. This enumerating system also utilized here. apple vinegar I, grape vinegar I and pomegranate vinegar I belong to Kuhne Vinegar Factory, pomegranate vinegar II belongs to Yedier Vinegar Factory, and grape vinegar II, apple vinegar II belong to Nahita Natural Fermentation Factory. Phenolic compounds of the samples were measured with HPLC-PDA and gained peaks compared with peaks of phenolic standard which had been identified before the analysis. Measured phenolics are shown at Table 4.24 and results of samples were given as mg /100 mL samples.

Table 4.24: The comparasion of phenolic compounds of different types of vinegar

	Gallic Acid	Protocatechuic Acid	<i>p</i>-Hydroxybenzoic Acid	Catechin
Apple Vinegar I	0.92±0.05	0.23±0.01	0.34±0.05	2.27±0.01
Grape Vinegar I	5.16±0.08	0.43±0.01	0.45±0.00	N.D.
Pomegranate Vinegar I	7.94±0.18	3.51±0.03	6.05±0.23	12.57±1.18
Balsamic Vinegar	83.02±0.67	N.D.	N.D.	N.D.
Blueberry Vinegar	60.08±12.93	18.74±0.12	N.D.	N.D.
Rosehip Vinegar	0.26±0.00	0.48±0.11	0.76±0.05	0.64±0.37
Pomegranate Vinegar II	0.10±0.00	0.07±0.00	0.27±0.00	0.06±0.00
Grape Vinegar II	2.76±0.09	0.28±0.00	2.26±0.15	9.69±0.44
Gilaburu Vinegar	0.98±0.52	0.28±0.00	6.09±5.81	2.32±1.90
Apple Vinegar II	0.45±0.07	0.45±0.21	0.30±0.00	N.D.
Lemon Vinegar	0.21±0.21	0.21±0.08	0.75±0.61	0.22±0.04
Blackberry Vinegar	0.11±0.02	0.31±0.04	1.49±0.30	0.55±0.29
Artichoke Vinegar	0.45±0.24	0.29±0.04	4.67±0.27	0.35±0.00
Mulberry Vinegar	0.50±0.02	0.58±0.06	0.74±0.01	0.47±0.13
Rice Vinegar	0.34±0.01	0.41±0.01	0.52±0.06	0.36±0.04
Apricot Vinegar	0.38±0.00	0.26±0.01	0.64±0.09	0.13±0.01
Date Vinegar	0.52±0.03	0.41±0.05	0.66±0.03	N.D.
Howthorn Vinegar	0.10±0.10	0.54±0.00	0.62±0.00	0.29±0.00

*Results were given in mg /100 mL samples for all.

**N.D. : Not dedected.

Balsamic vinegar had the highest gallic acid concentration in all samples whereas its protocatechuic acid, *p*-hydroxybenzoic acid and catechin concentration were not detected. Balsamic vinegar was followed by blueberry vinegar with 60.08±12.93 mg gallic acid/100 mL. Blueberry vinegar also had the arised protocatechuic acid level with 18.74±0.12 mg/100 mL. Gilaburu vinegar and pomegranate vinegar I had more

p-hydroxybenzoic acid than other vinegars. Besides this catechin concentration level of pomegranate vinegar I and grape vinegar II were clearly left behind other vinegars.

In addition to these, comparasion of same vinegars from different factory is also possible here too. Apple vinegar I had higher gallic acid, *p*-hydroxybenzoic acid and catechin concentration while apple vinegar II had more protocatechuic acid concentration. Taking into account grape vinegar I and grape vinegar II, it could observed easily gallic acid and protocatechuic concentration level were higher in grape vinegar I while , *p*-hydroxybenzoic acid and catechin concentration were less than grape vinegar II. Noticeable differences were also found between pomegranate vinegar I and pomegranate vinegar II.

5. CONCLUSIONS AND RECOMMENDATIONS

Vinegar was known since old Oriental civilizations and was employed as a poor man's drink and later as a remedy in ancient Greece and Rome. It is still the most important single flavoring used to provide or enhance the sour, acidic taste of food . It is commonly used as an ingredient in the food systems and it is a good solvent for the essential oils of herbs and spices and has been a ubiquitous sauce ingredient throughout history. Besides these properties, vinegar has some additional health effects; such as promoting recovery from exhaustion, regulating blood glucose level, blood pressure, aiding digestion, stimulating the appetite, and promoting calcium absorption .

The aim of this study was firstly to determine the effect of processing on total phenolic and total flavonoid contents as well as total antioxidant capacity of the selected vinegars. For this purpose, samples collected from different steps of vinegar processing were analyzed for their TFC, TPC, and TAC using ABTS, CUPRAC, DPPH, and FRAP methods. The changes in individual phenolics of the vinegar processing samples were also monitored by HPLC. In addition, the second objective of this present work was to evaluate the *in vitro* bioaccessibility of antioxidants in some selected vinegars by using the same spectrophotometric methods described above. Finally, comparisons in between the contents and profiles of antioxidants in different types of vinegars was also performed within this study.

Samples from different steps of grape and apple vinegar processing were collected; including grape wine (GW), raw grape vinegar (not decanted and not filtered) (RGV), decanted grape vinegar (DGV), decanted and filtered grape vinegar (DFGV), and final grape vinegar (FGV) samples for grape vinegar processing, and apple juice concentrate (AJC), apple wine (AW), decanted apple vinegar (DAV), decanted and filtered apple vinegar (DAV), and final apple vinegar product (FAV) for apple vinegar processing. The general trend, for the results obtained on fresh weight basis,

was a continuous decrease from the starting material to the end product, whereas this was not observed with the results obtained on dry weight basis.

When the results were examined for each successive step, the values observed for DGV, DFGV, and FGV in grape vinegar processing, and for DAV, DFAV, and FAV in apple vinegar processing, were found not to be statistically different. Furthermore, although there was a dilution step included in processing of decanted and filtered vinegar samples to the final vinegars, the values obtained for the samples from these different steps were found to be similar to each other (on dry weight basis), differentiating from what was expected. This could be explained with the up-regulation of the antioxidant levels in the final product with the additional use of sodium metabisulfite as an antioxidant.

Although the antioxidant levels in apple vinegar was lower in comparison to the grape, pomegranate, and balsamic vinegar, the recovery of apple antioxidants were found to be the highest in *in vitro* bioaccessibility assay. On the other hand, when the overall bioaccessible amounts of antioxidants were taken into consideration, it was observed to be the highest in balsamic vinegar since it had higher initial levels.

This study also enabled the comparison of different methods applied during processing of different vinegars. Using micro-filtration as a filtration process or the type of barrels used were found to cause differences between samples which mentioned before several authors. The traditional vinegar processing method used in apple and grape vinegar production was determined to give rise to more than 2-fold higher antioxidant values in comparison to the acetator system. However, this was not the case for pomegranate vinegar. In contrast to the results obtained for apple and grape vinegars, the acetator system used in pomegranate vinegar processing was found to lead to more than 3-fold and 2-fold higher TFC and TPC values, respectively, when compared to the values obtained with traditional method. This could be related to the addition of sodium metabisulfite as an antioxidant in pomegranate vinegar processing using acetator system. Another possible reason might be the different dry matter contents in samples (13.5° Brix for pomegranate vinegar produced with acetator and 2.3° Brix for pomegranate vinegar produced

with traditional method). In the comparison of this vinegars fresh weight basis results were utilized.

In addition to the determination of the effect of processing, this study also monitored and compared the contents of antioxidants in 18 different vinegar samples. Among the analyzed vinegar samples, balsamic vinegar gave the highest values for TPC, TFC, and TAC -determined using CUPRAC and FRAP methods-, followed by rosehip vinegar. The differences between different types of vinegar samples could be generally linked to the differences between the raw fruit materials.

Balsamic vinegar had the highest gallic acid concentration in all samples according to HPLC-PDA results, whereas its protocatechuic acid, *p*-hydroxybenzoic acid and catechin concentration were not detected. Blueberry vinegar also had the arised protocatechuic acid level, gilaburu vinegar and pomegranate vinegar I had more *p*-hydroxybenzoic acid than other vinegars. Besides this catechin concentration level of pomegranate vinegar I and grape vinegar II were clearly left behind other vinegars. In addition to these, apple vinegar I had higher gallic acid, *p*-hydroxybenzoic acid and catechin concentration while apple vinegar II had more protocatechuic acid concentration. Taking into account grape vinegar I and grape vinegar II, it could observed easily gallic acid and protocatechuic concentration level were higher in grape vinegar I while , *p*-hydroxybenzoic acid and catechin concentration were less than grape vinegar II.

This study provides valuable data on the antioxidant properties of various types of vinegars as well as *in vitro* bioaccessibility of vinegar antioxidants, and could be a basis for the further studies that could be extended with the use several other vinegar, wine and juice samples to provide additional data. In addition to this, *in vitro* bioaccessibility methods cannot directly predict the human *in vivo* conditions. Further studies would also focus on this issue..

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APPENDIX A

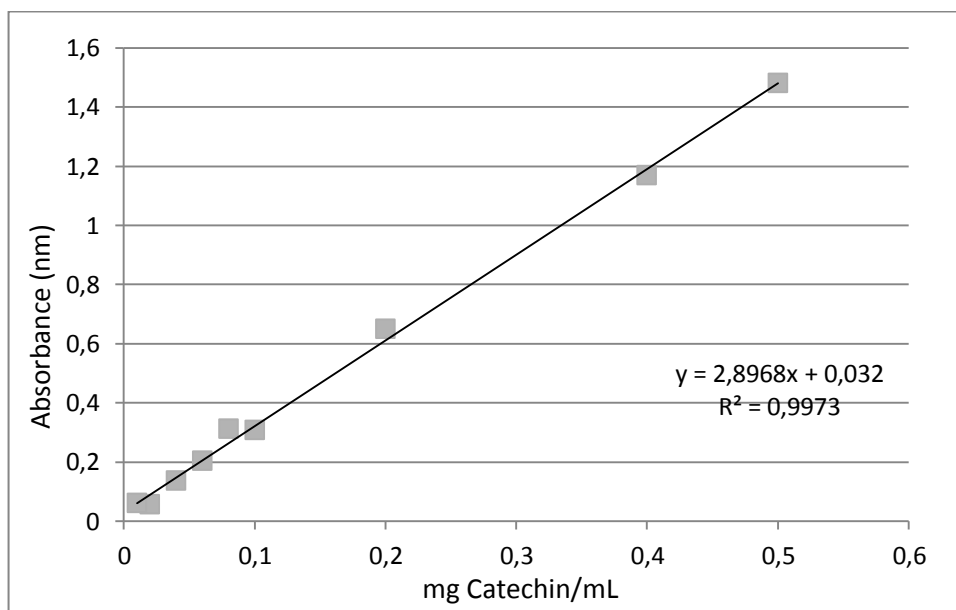


Figure A. 1: Standart calibration curve for total flavonoid content analysis

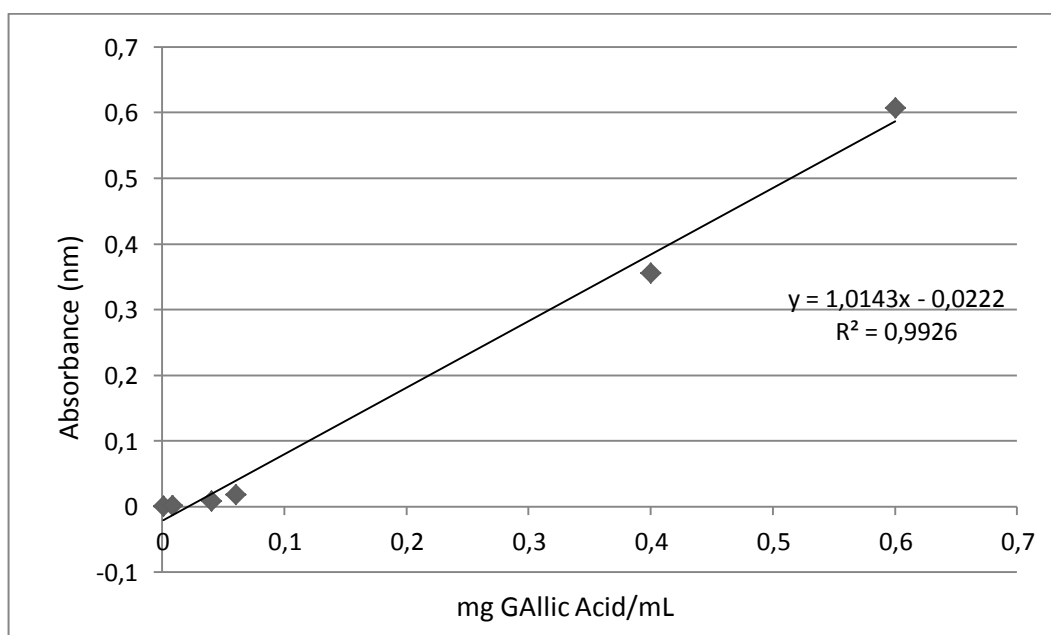


Figure A. 2: Standart calibration curve for total phenolic content analysis

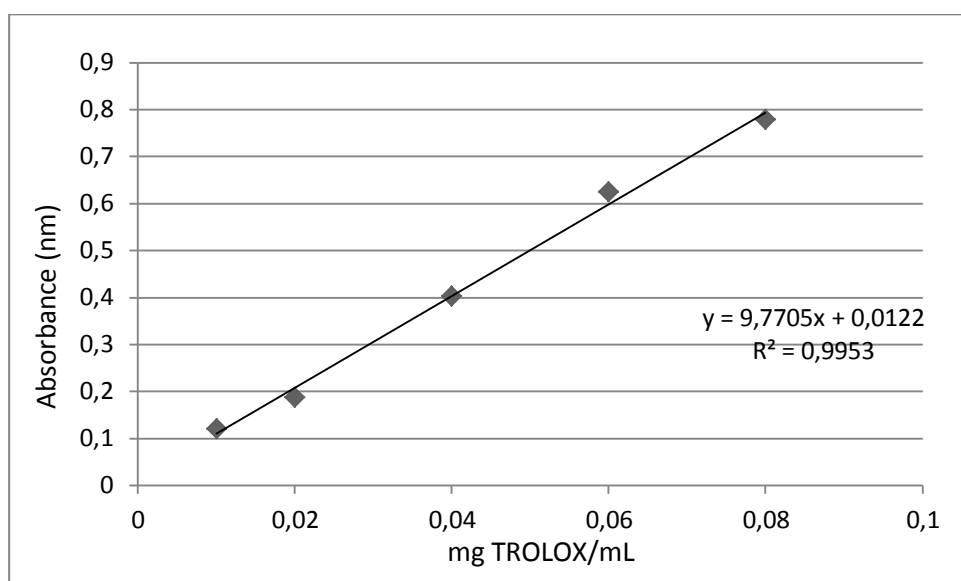


Figure A. 3: Standart calibration curve of TROLOX for ABTS analysis

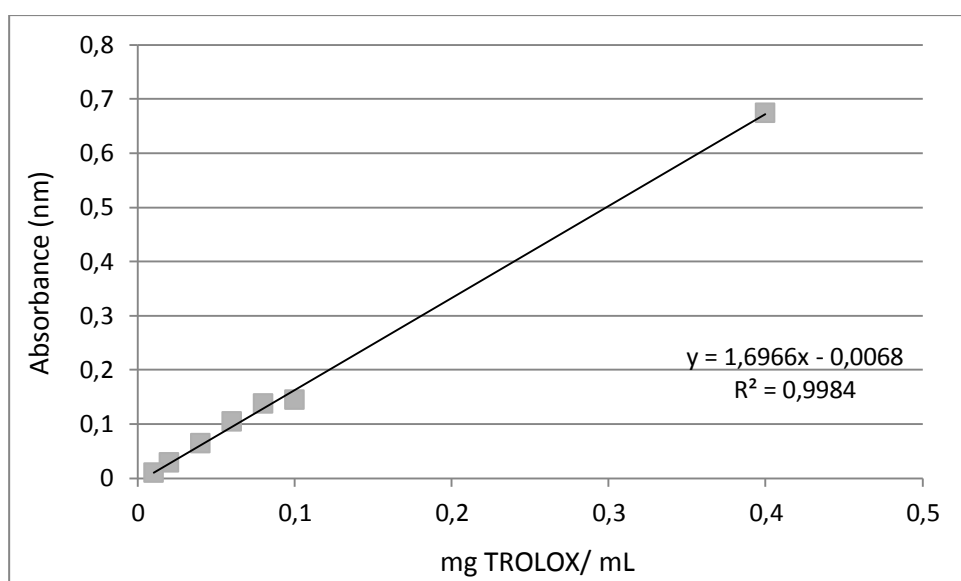


Figure A. 4: Standart calibration curve of TROLOX for CUPRAC analysis

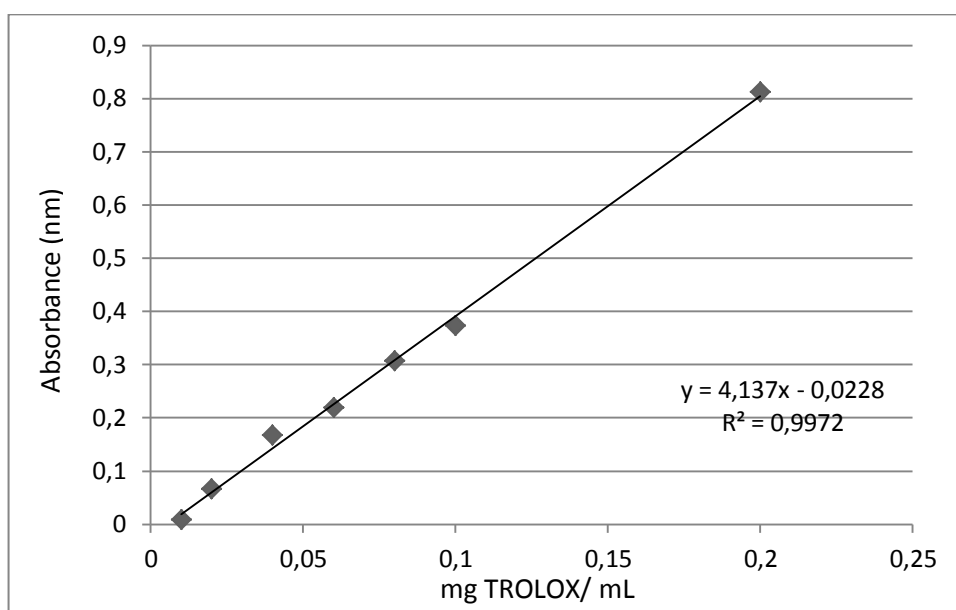


Figure A. 5: Standart calibration curve of TROLOX for DPPH analysis

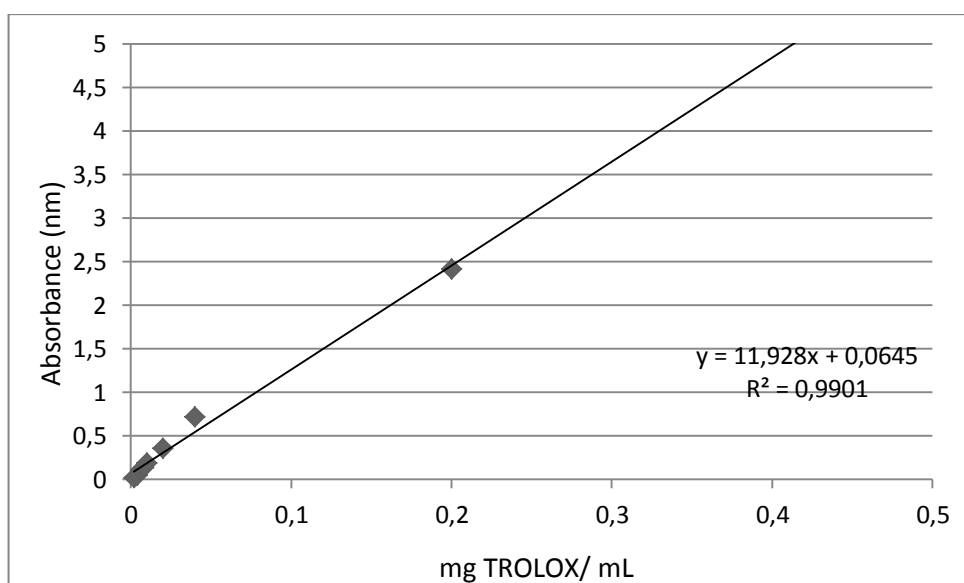


Figure A. 6: Standart calibration curve of TROLOX for FRAP analysis

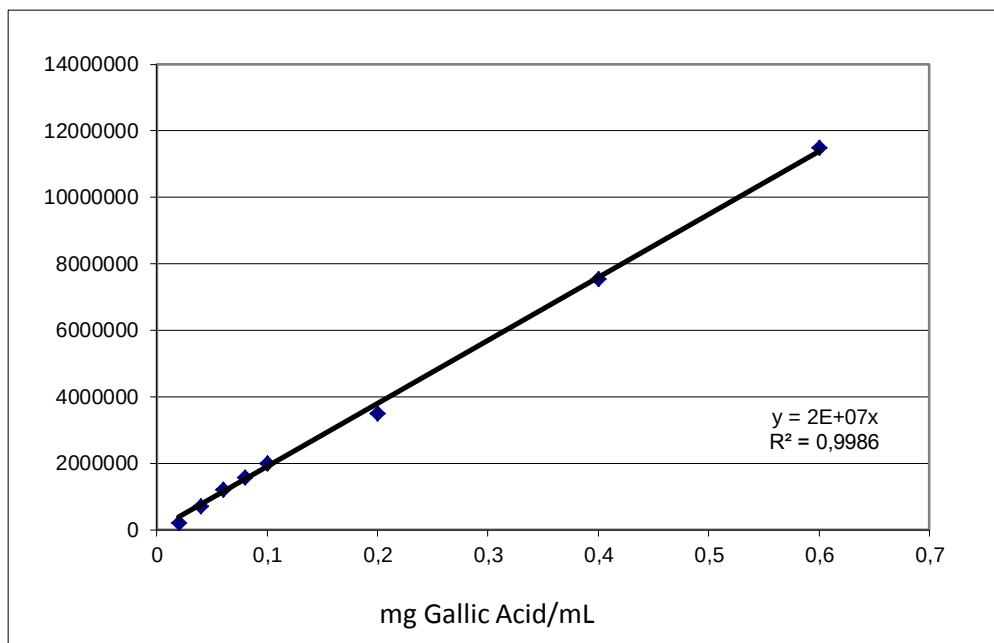


Figure A. 7: Standard calibration curve of *gallic* acid for HPLC

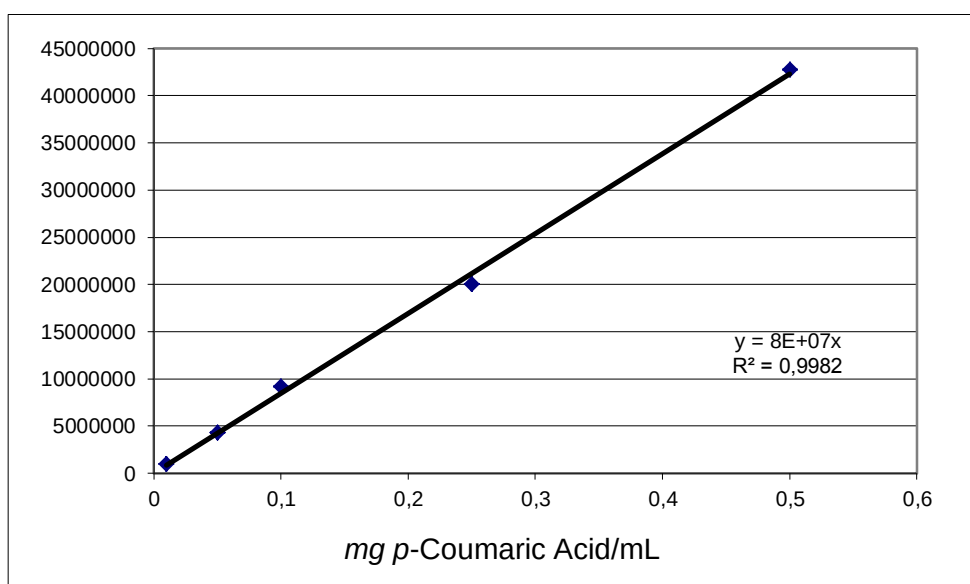


Figure A. 8: Standard calibration curve of *p*-coumaric acid for HPLC

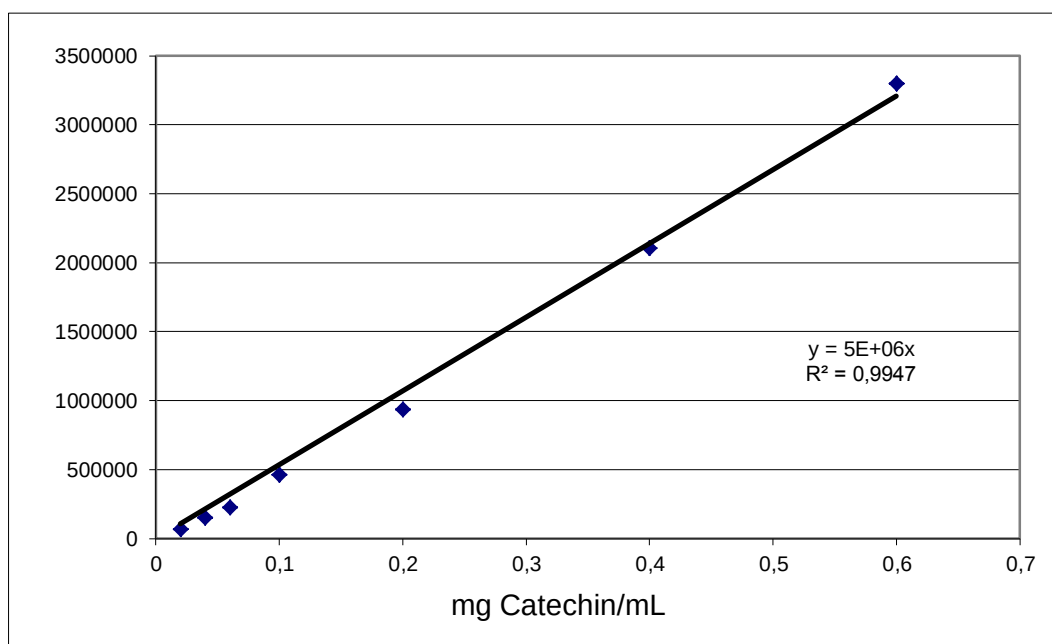


Figure A. 9: Standard calibration curve of catechin for HPLC

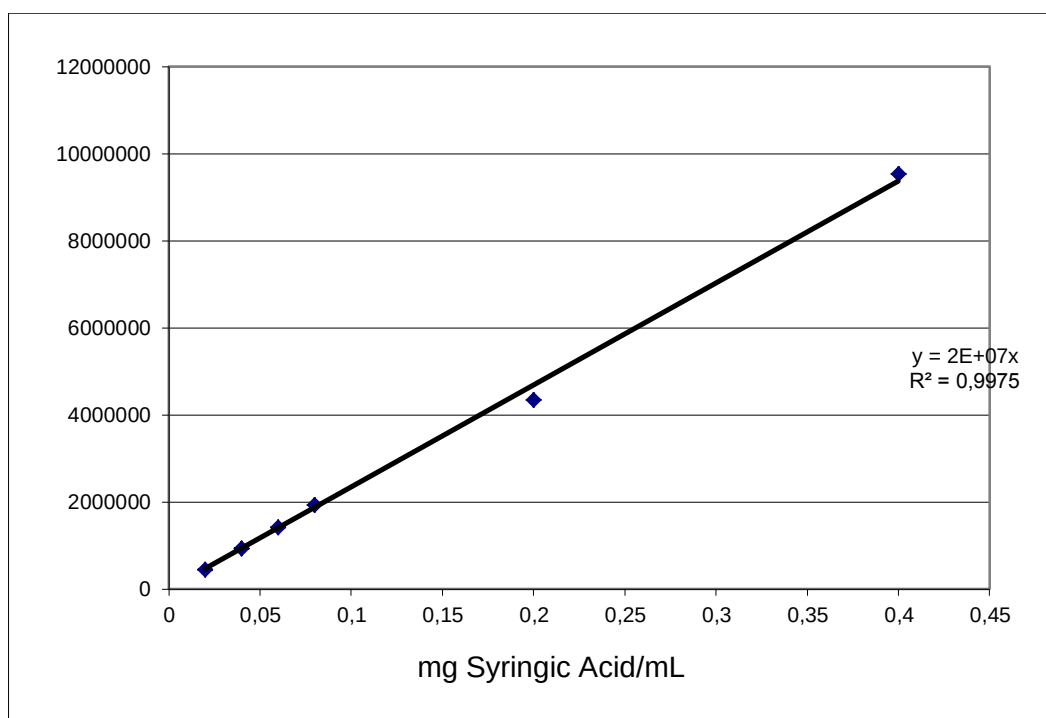


Figure A. 10: Standard calibration curve of syringic acid for HPLC

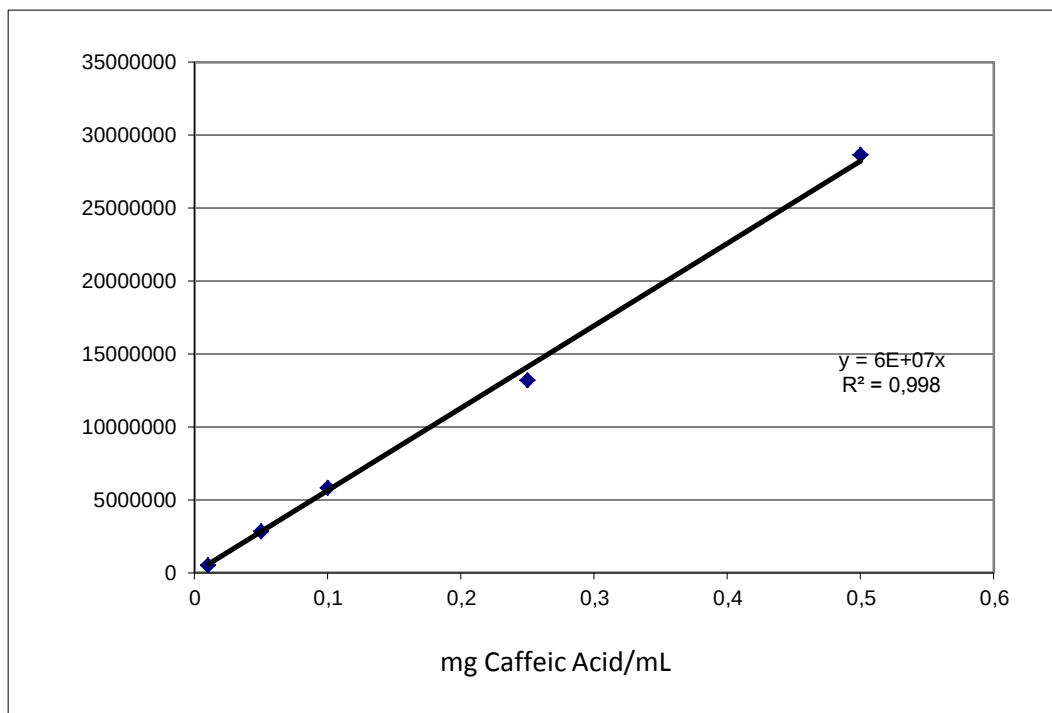


Figure A. 11: Standard calibration curve of caffeic acid for HPLC

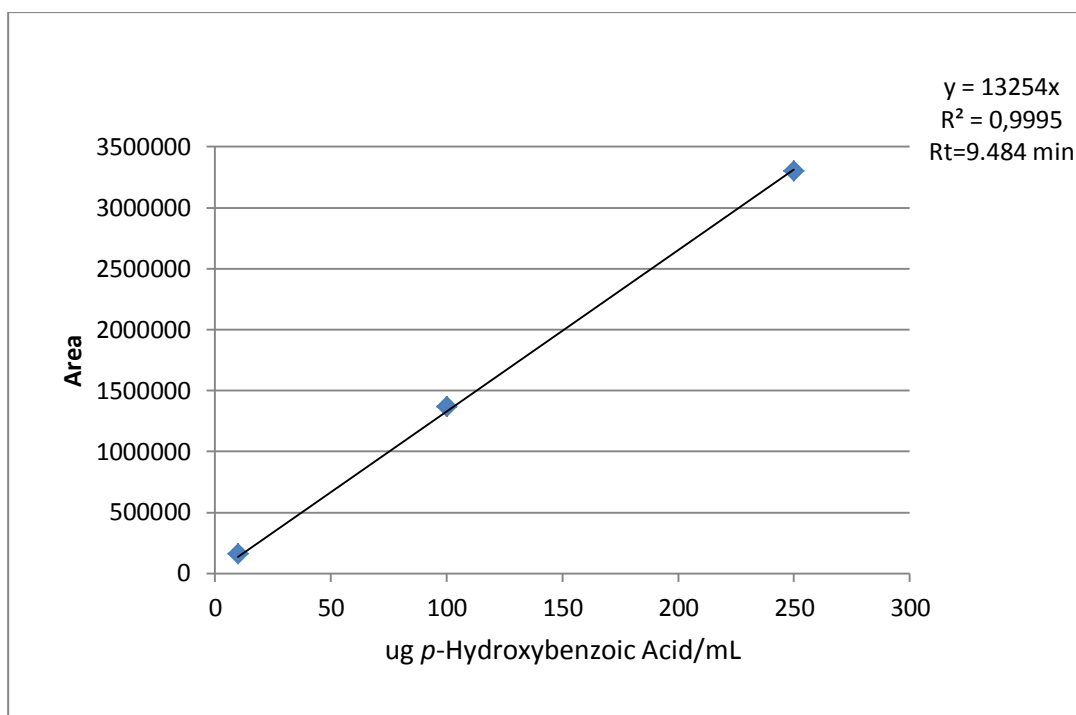


Figure A. 12: : Standard calibration curve of *p*-hydroxybenzoic acid for HPLC

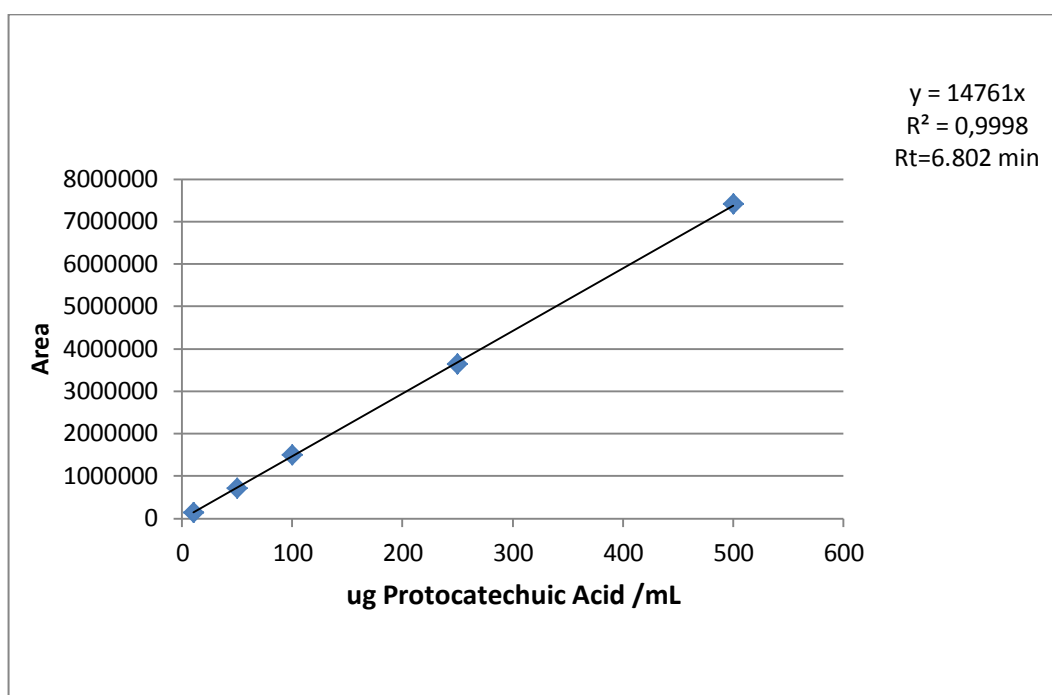


Figure A. 13: Standard calibration curve of protocatechuic acid for HPLC

APPENDIX B

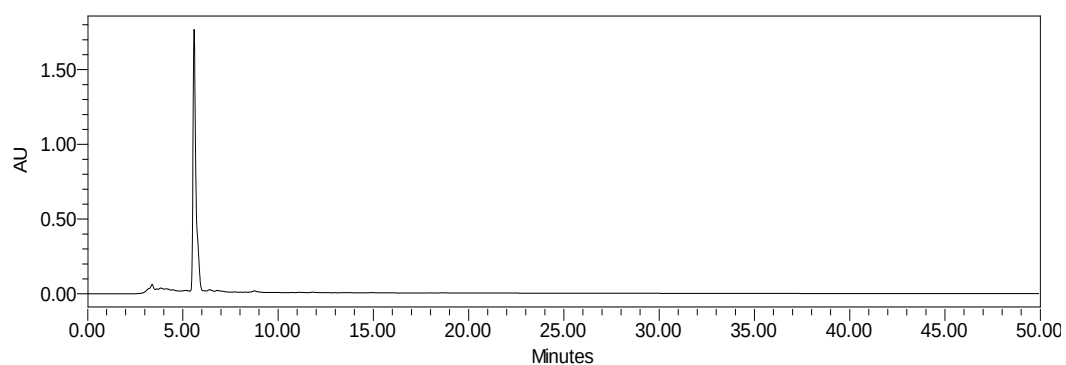


Figure B.1: Representative HPLC chromatograms of grape wine sample at 280 nm.

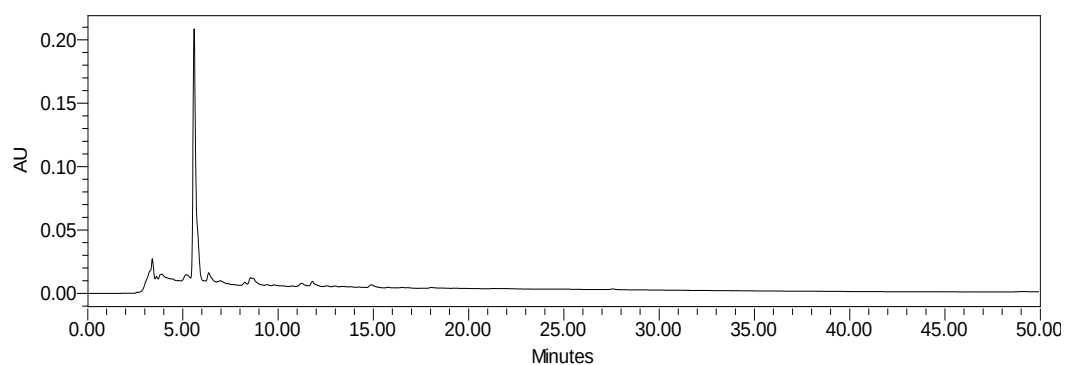


Figure B.2: Representative HPLC chromatograms of grape wine sample at 312 nm.

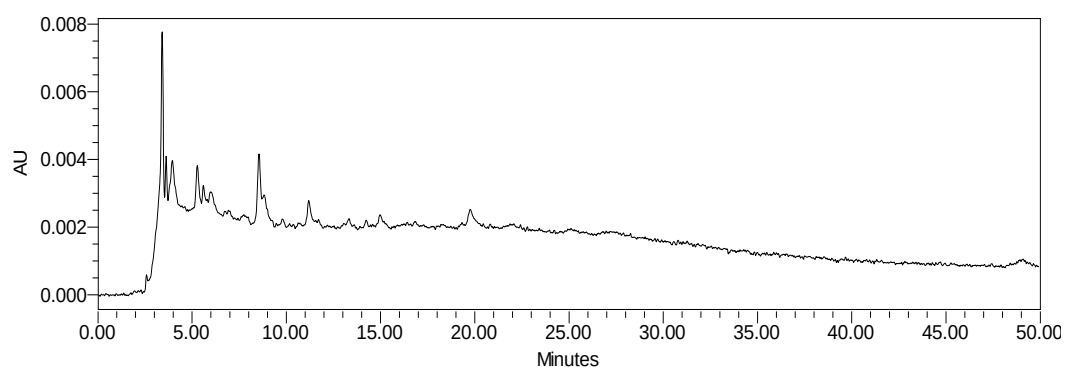


Figure B.3: Representative HPLC chromatograms of grape wine sample at 360 nm.

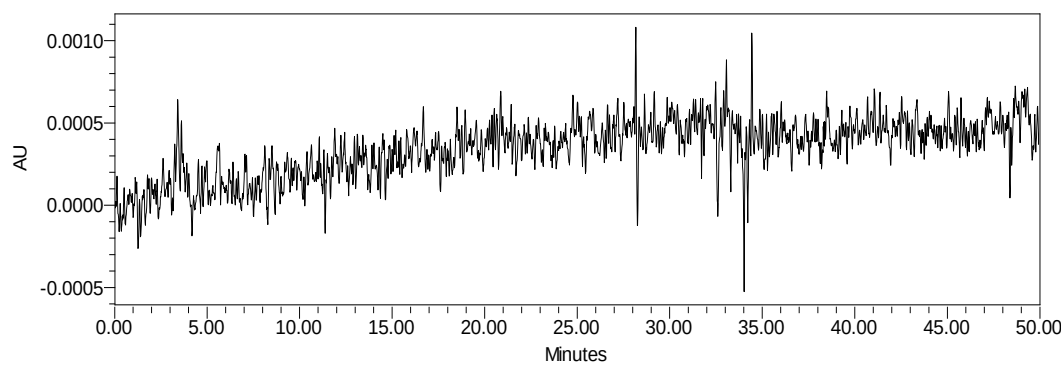


Figure B.4: Representative HPLC chromatograms of grape wine sample at 520 nm.

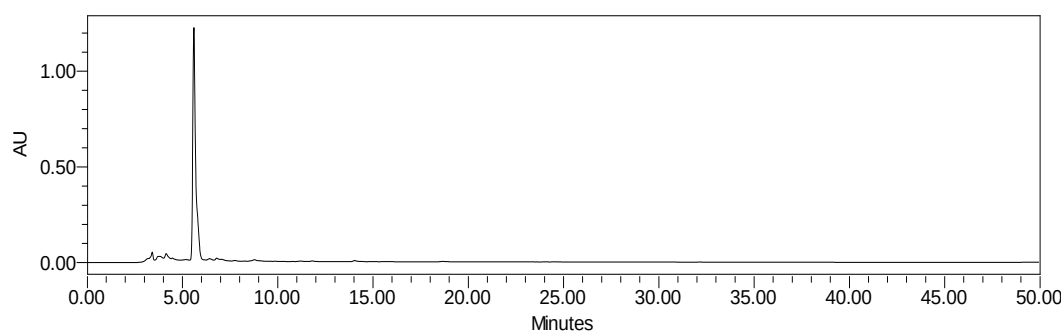


Figure B.5: Representative HPLC chromatograms of raw grape vinegar sample at 280 nm.

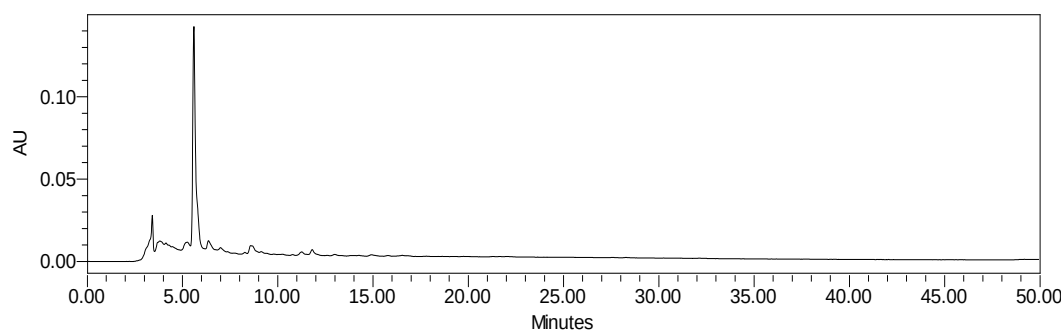


Figure B.6: Representative HPLC chromatograms of raw grape vinegar sample at 312 nm.

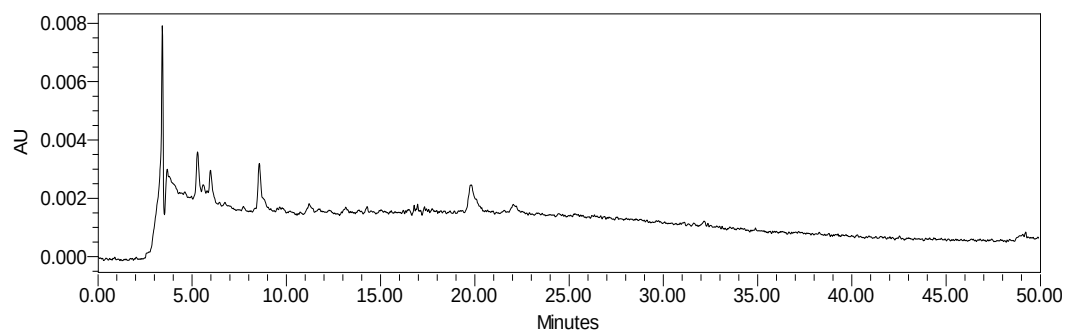


Figure B.7: Representative HPLC chromatograms of raw grape vinegar sample at 360 nm.

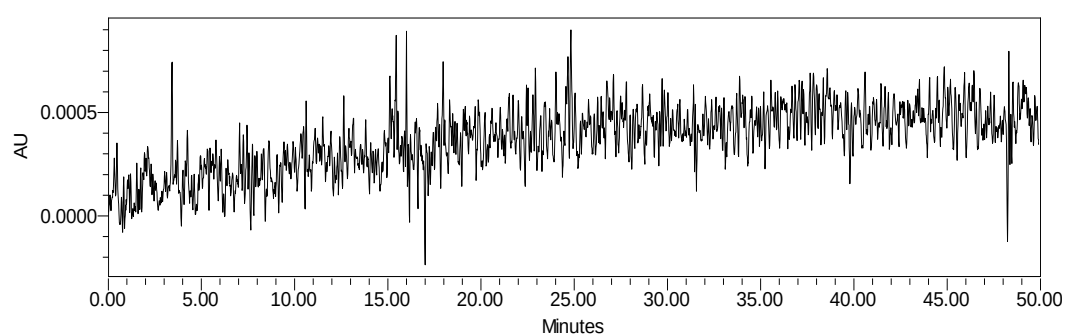


Figure B.8: Representative HPLC chromatograms of raw grape vinegar sample at 520 nm.

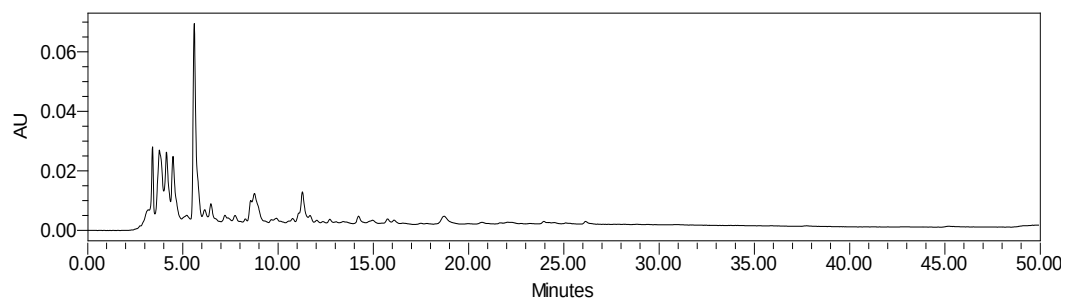


Figure B.9: Representative HPLC chromatograms of decanted grape vinegar sample at 280 nm.

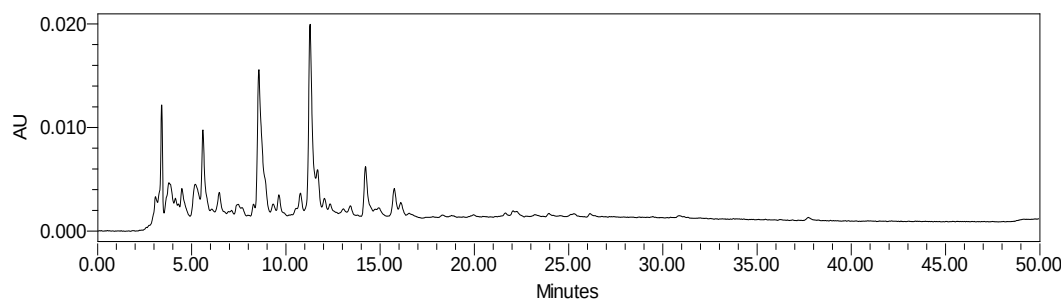


Figure B.10: Representative HPLC chromatograms of decanted grape vinegar sample at 312 nm.

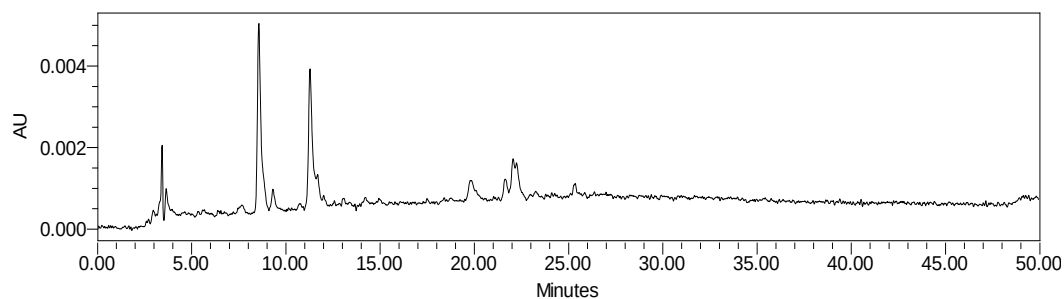


Figure B.11: Representative HPLC chromatograms of decanted grape vinegar sample at 360 nm.

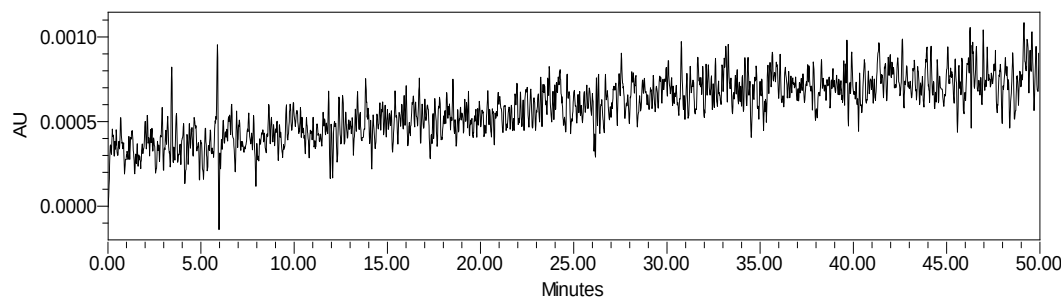


Figure B.12: Representative HPLC chromatograms of decanted grape vinegar sample at 520 nm.

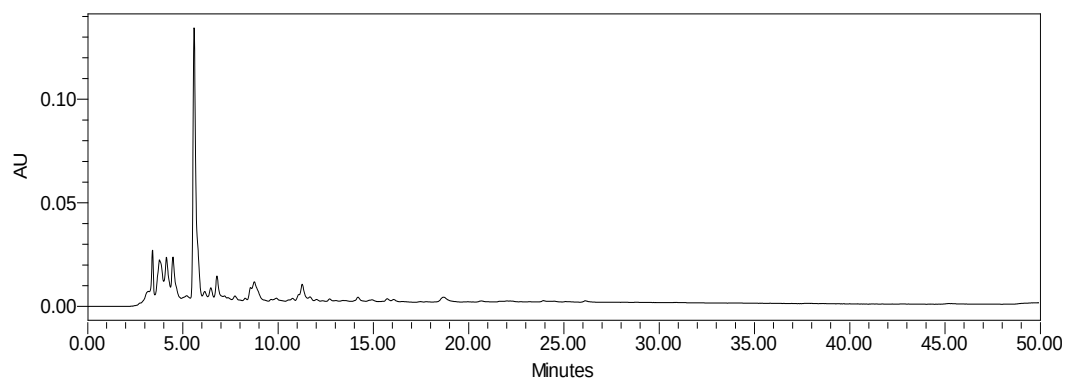


Figure B.13: Representative HPLC chromatograms of decanted and filtered grape vinegar sample at 280 nm.

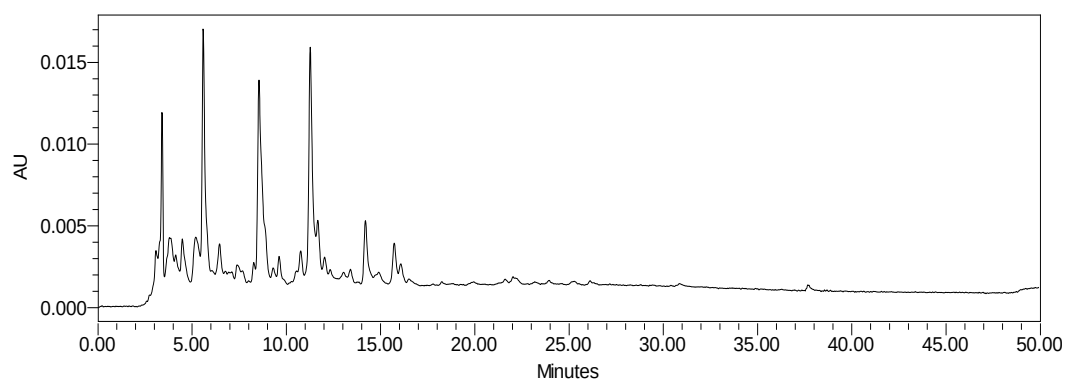


Figure B.14: Representative HPLC chromatograms of decanted and filtered grape vinegar sample at 312 nm.

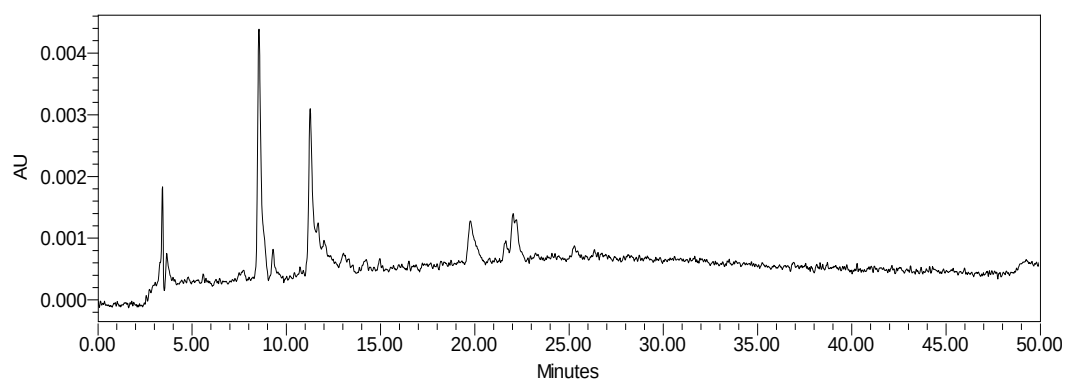


Figure B.15: Representative HPLC chromatograms of decanted and filtered grape vinegar sample at 360 nm.

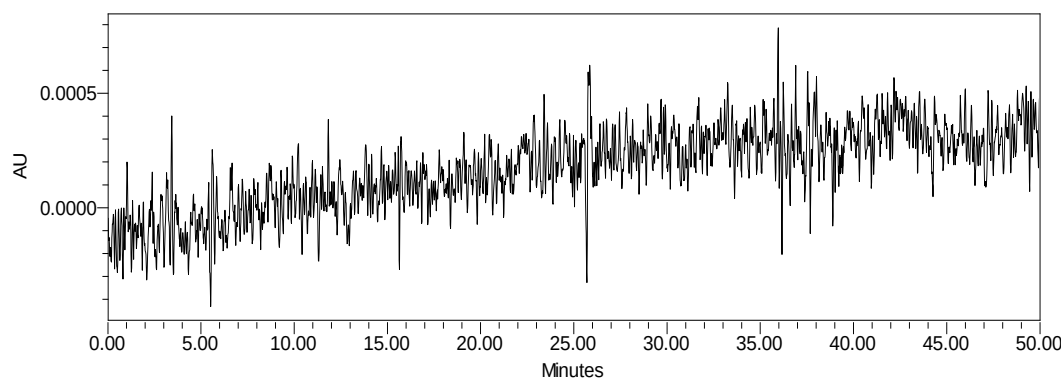


Figure B.16: Representative HPLC chromatograms of decanted and filtered grape vinegar sample at 520 nm.

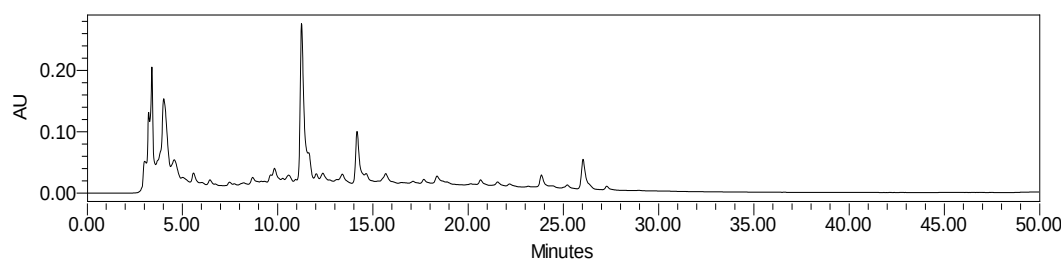


Figure B.17: Representative HPLC chromatograms of apple juice concentrate sample at 280 nm.

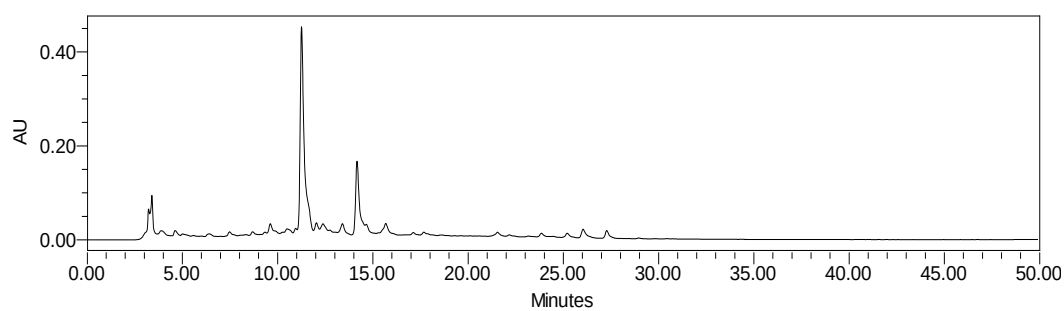


Figure B.18: Representative HPLC chromatograms of apple juice concentrate sample at 312 nm.

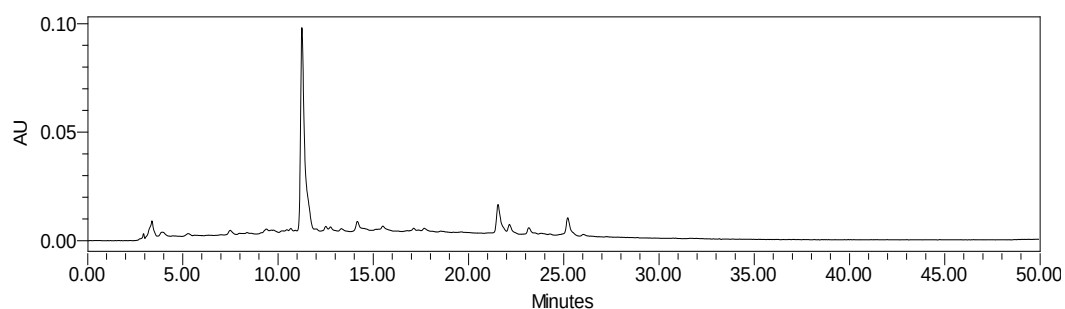


Figure B.19: Representative HPLC chromatograms of apple juice concentrate sample at 360 nm.

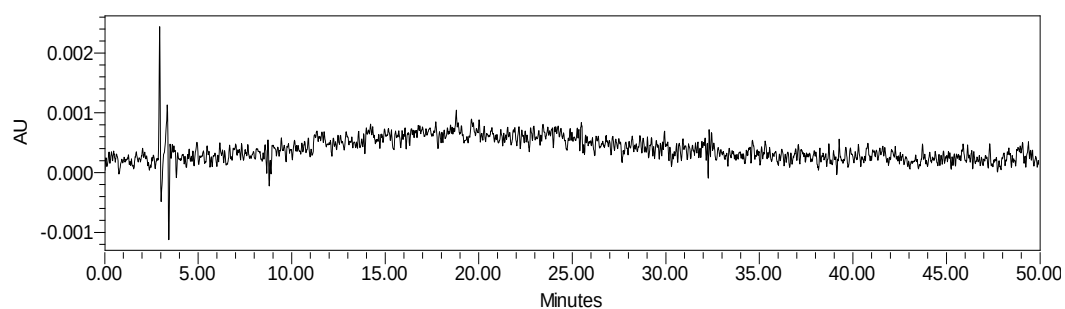


Figure B.20: Representative HPLC chromatograms of apple juice concentrate sample at 520 nm.

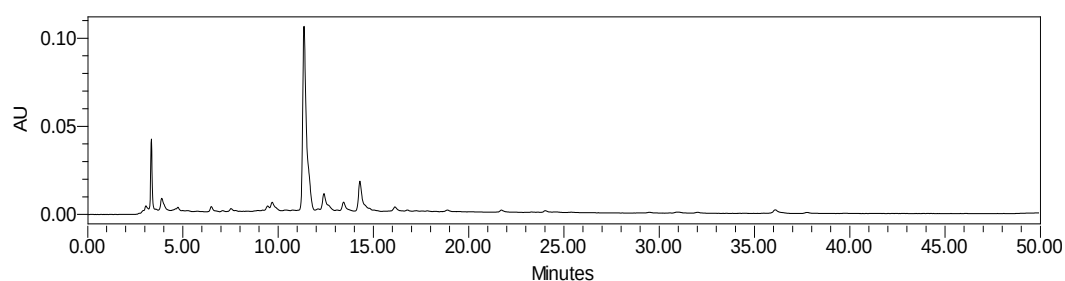


Figure B.21: Representative HPLC chromatograms of apple wine sample at 280 nm.

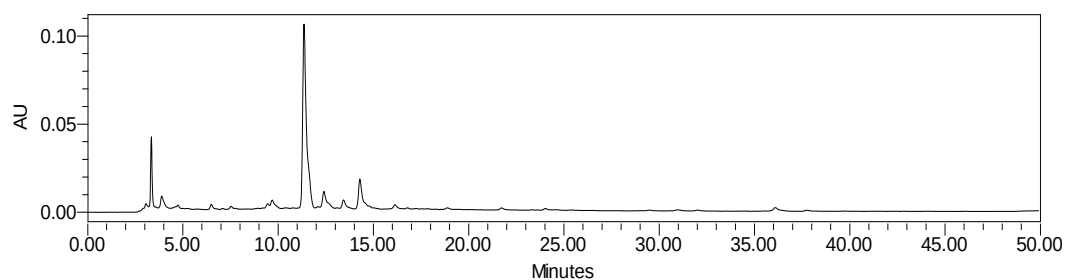


Figure B.22: Representative HPLC chromatograms of apple wine sample at 312 nm.

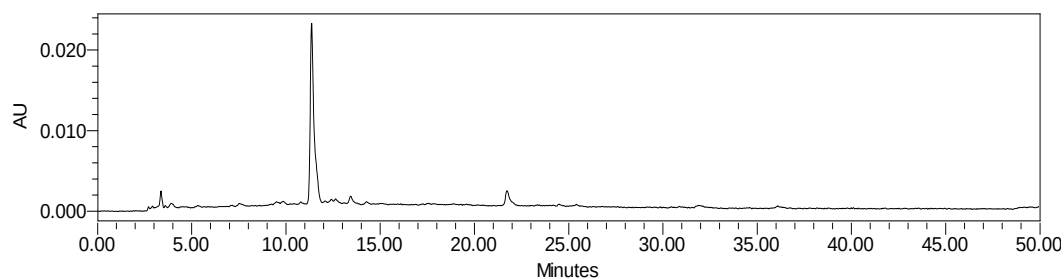


Figure B.23: Representative HPLC chromatograms of apple wine sample at 360 nm.

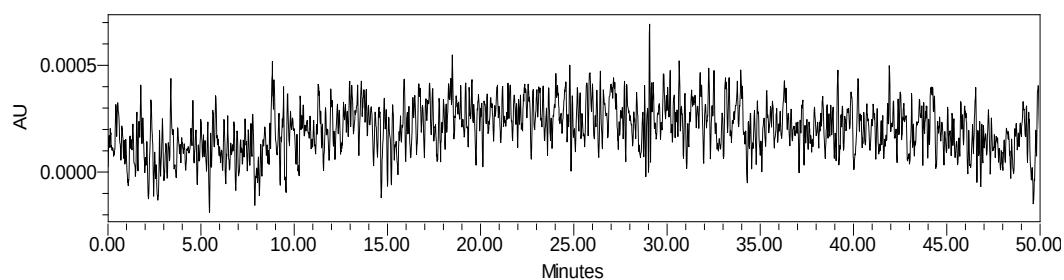


Figure B.24: Representative HPLC chromatograms of apple wine sample at 520 nm.

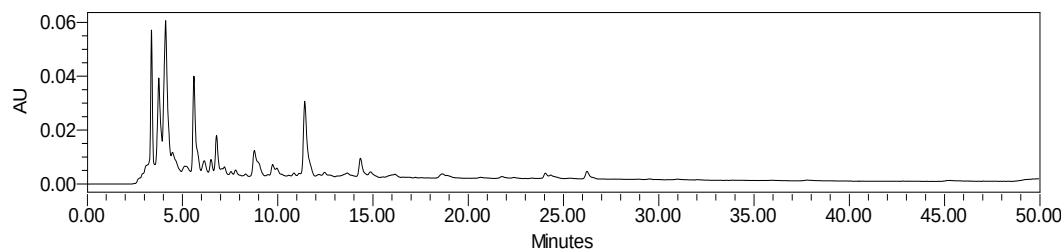


Figure B.25: Representative HPLC chromatograms of decanted apple vinegar sample at 280 nm.

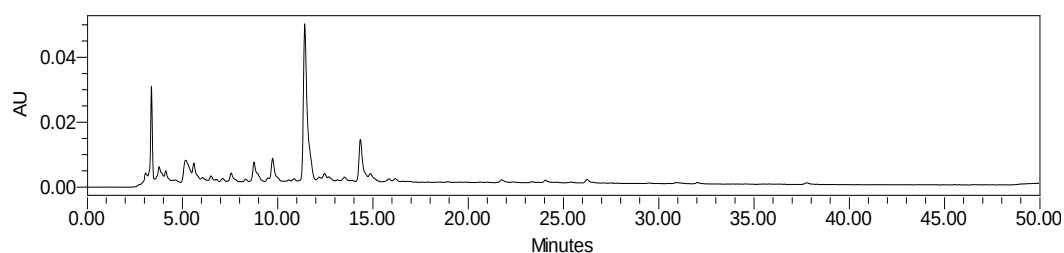


Figure B.26: Representative HPLC chromatograms of decanted apple vinegar sample at 312 nm.

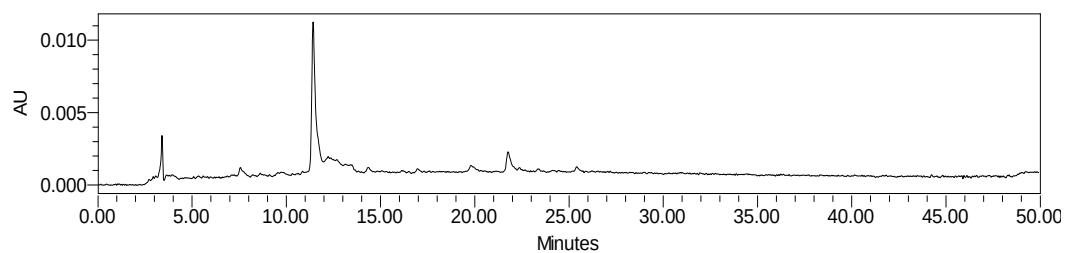


Figure B.27: Representative HPLC chromatograms of decanted apple vinegar sample at 360 nm.

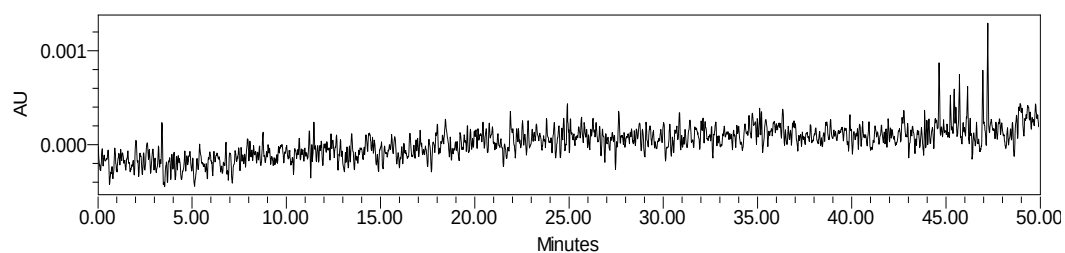


Figure B.28: Representative HPLC chromatograms of decanted apple vinegar sample at 520 nm.

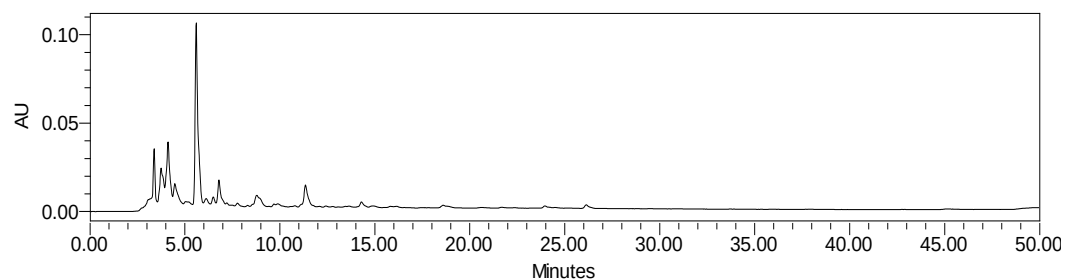


Figure B.29: Representative HPLC chromatograms of decanted and filtered apple vinegar sample at 280 nm.

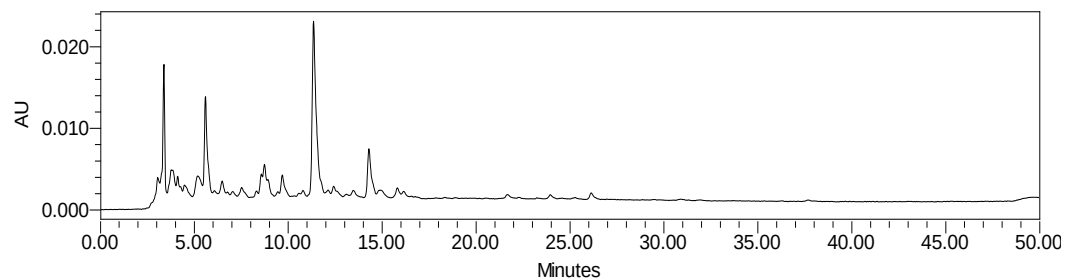


Figure B.30: Representative HPLC chromatograms of decanted and filtered apple vinegar sample at 312 nm.

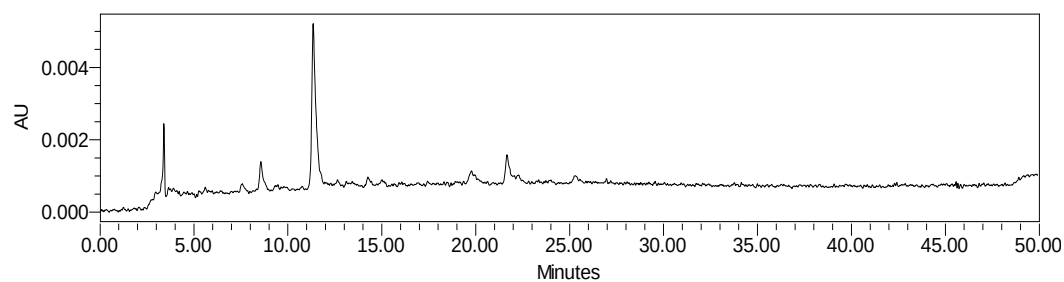


Figure B.31: Representative HPLC chromatograms of decanted and filtered apple vinegar sample at 360 nm.

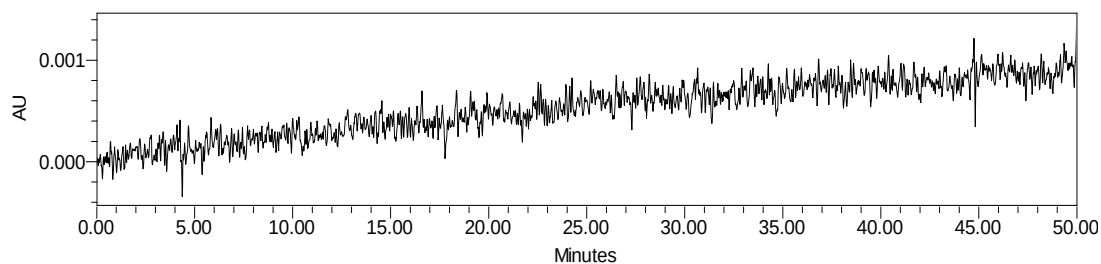


Figure B.32: Representative HPLC chromatograms of decanted and filtered apple vinegar sample at 520 nm.

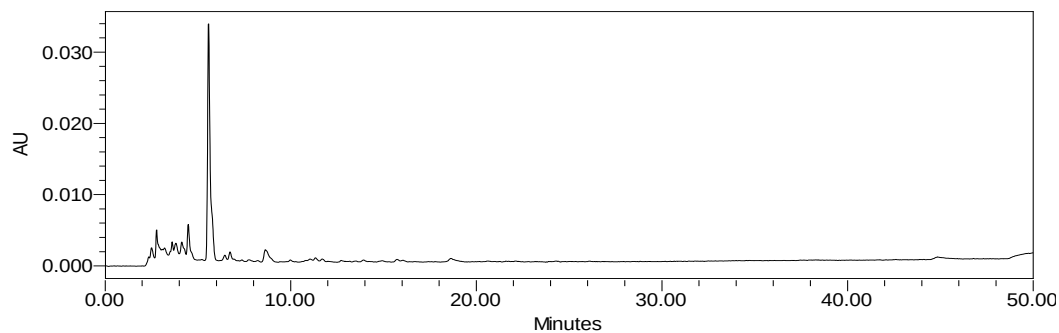


Figure B.33: Representative HPLC chromatograms of grape vinegar PG sample at 280 nm.

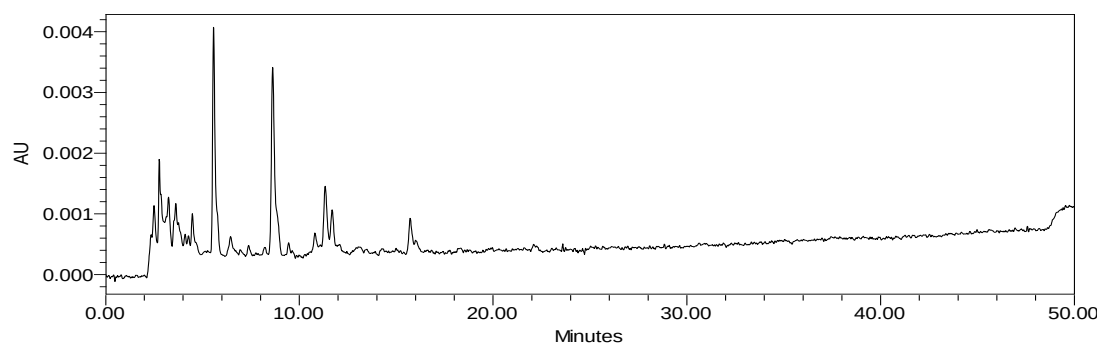


Figure B.34: Representative HPLC chromatograms of grape vinegar PG sample at 312 nm.

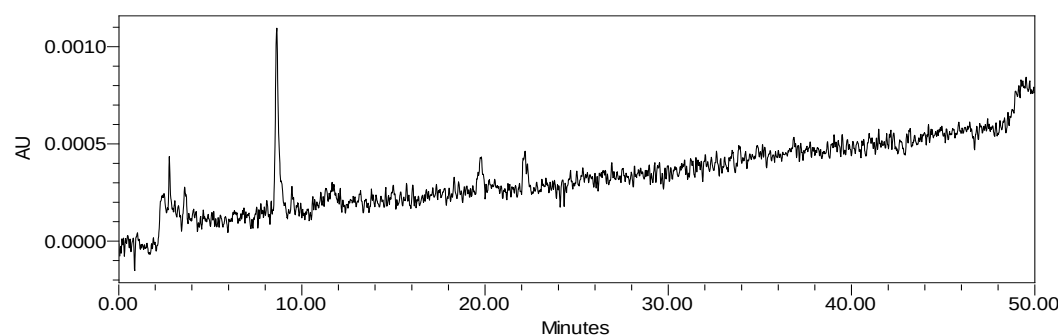


Figure B.35: Representative HPLC chromatograms of grape vinegar PG sample at 360 nm.

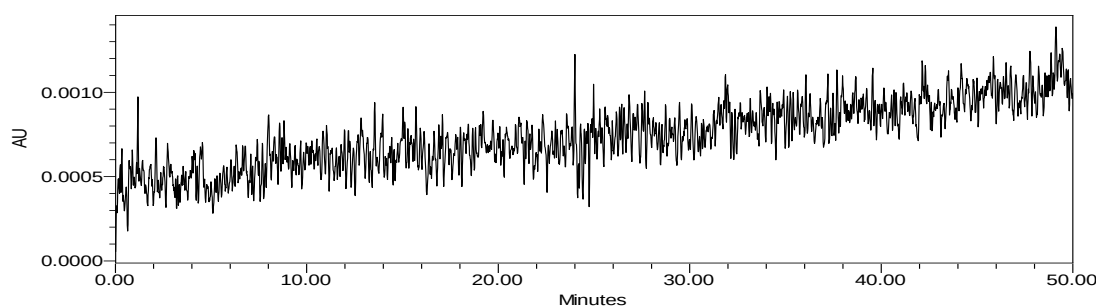


Figure B.36: Representative HPLC chromatograms of grape vinegar PG sample at 520 nm.

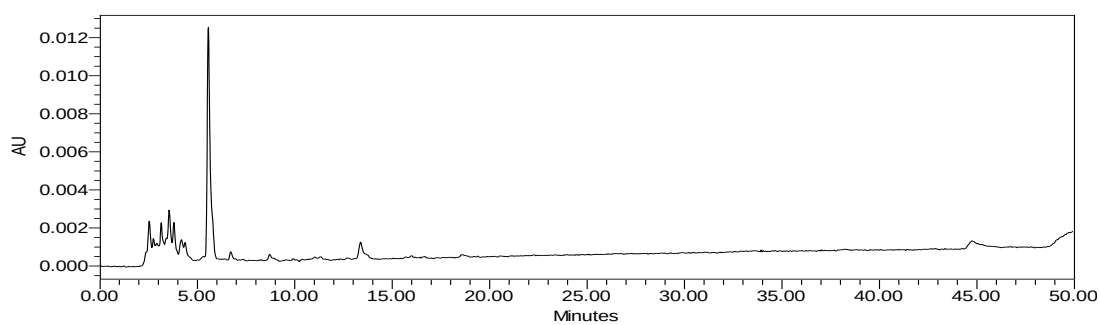


Figure B.37: Representative HPLC chromatograms of grape vinegar IN sample at 280 nm.

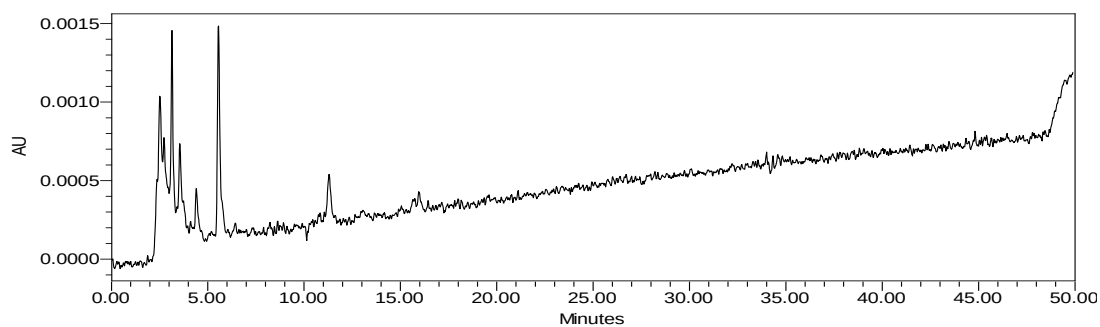


Figure B.38: Representative HPLC chromatograms of grape vinegar IN sample at 312 nm.

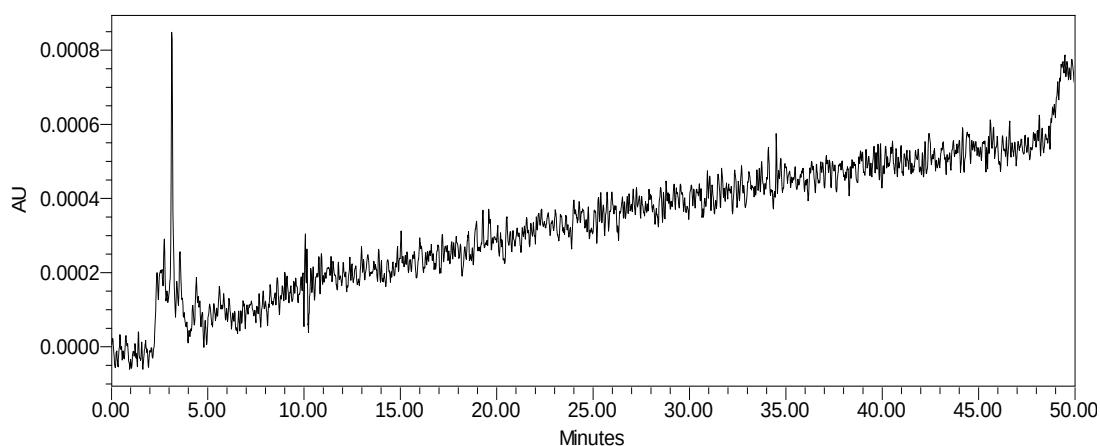


Figure B.39: Representative HPLC chromatograms of grape vinegar IN sample at 360 nm.

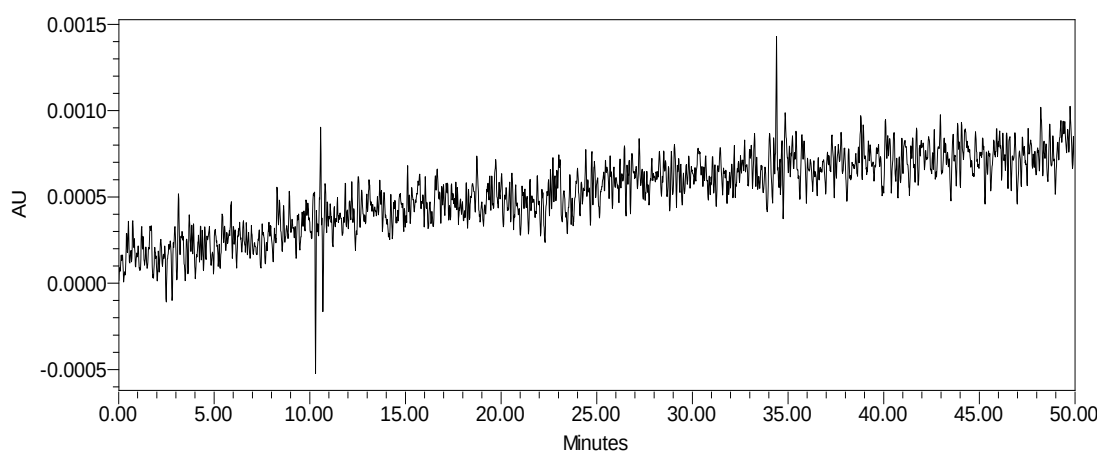


Figure B.40: Representative HPLC chromatograms of grape vinegar IN sample at 520 nm.

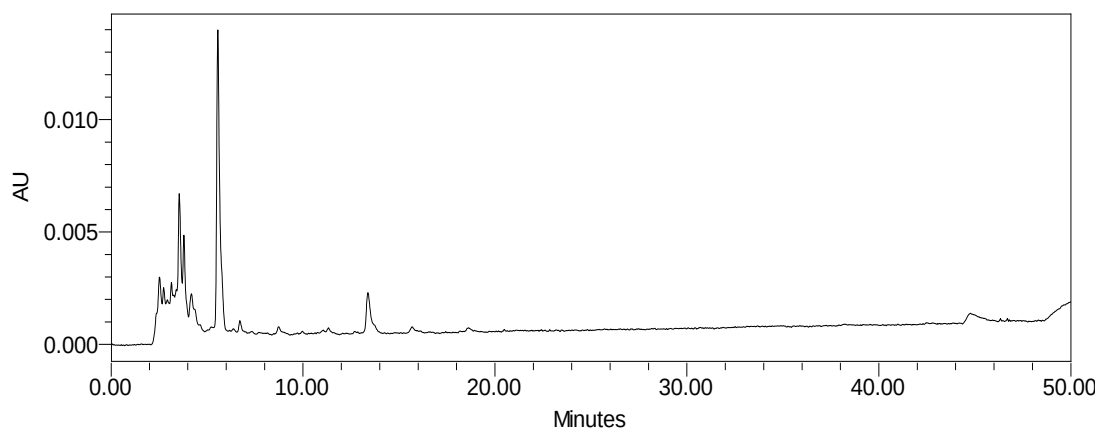


Figure B.41: Representative HPLC chromatograms of grape vinegar OUT sample at 280 nm.

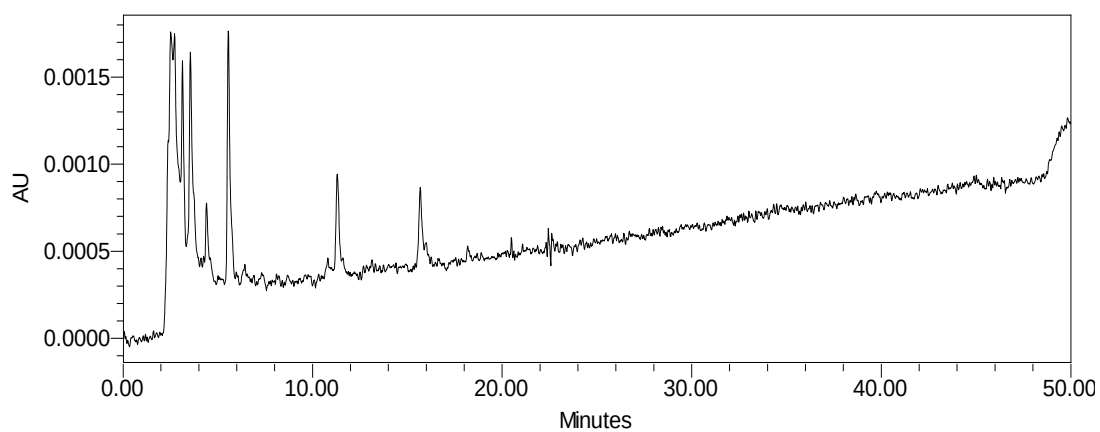


Figure B.42: Representative HPLC chromatograms of grape vinegar OUT sample at 312 nm.

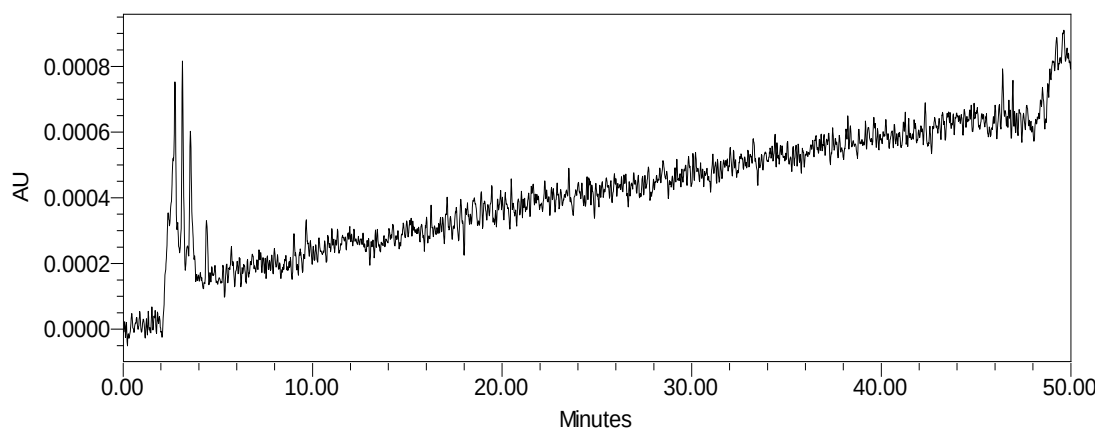


Figure B.43: Representative HPLC chromatograms of grape vinegar OUT sample at 360 nm.

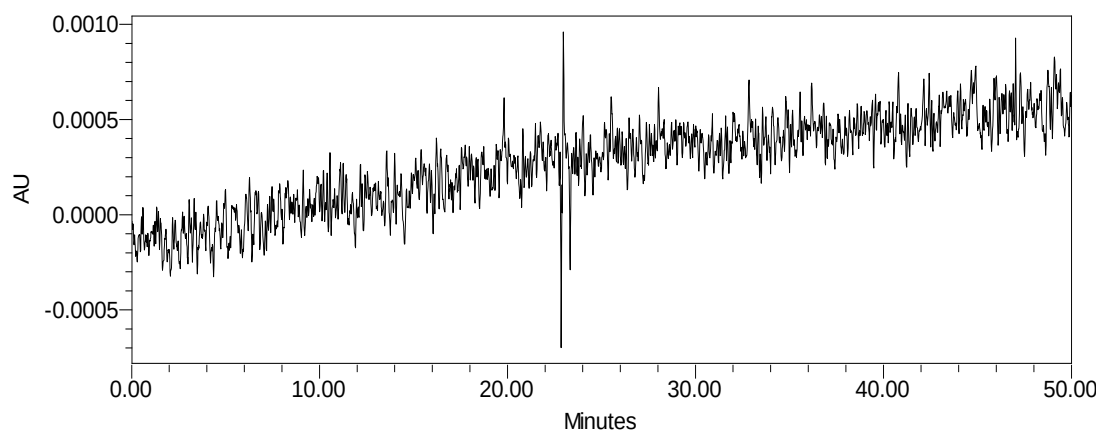


Figure B.44: Representative HPLC chromatograms of grape vinegar OUT sample at 520 nm.

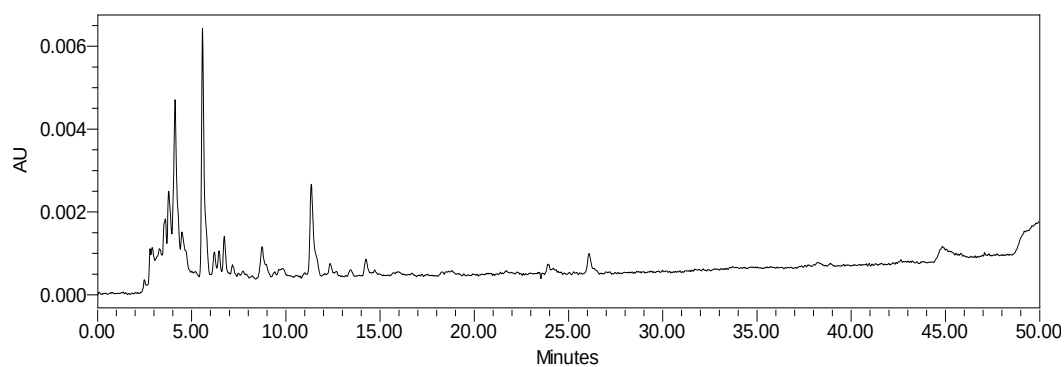


Figure B.45: Representative HPLC chromatograms of apple vinegar PG sample at 280 nm.

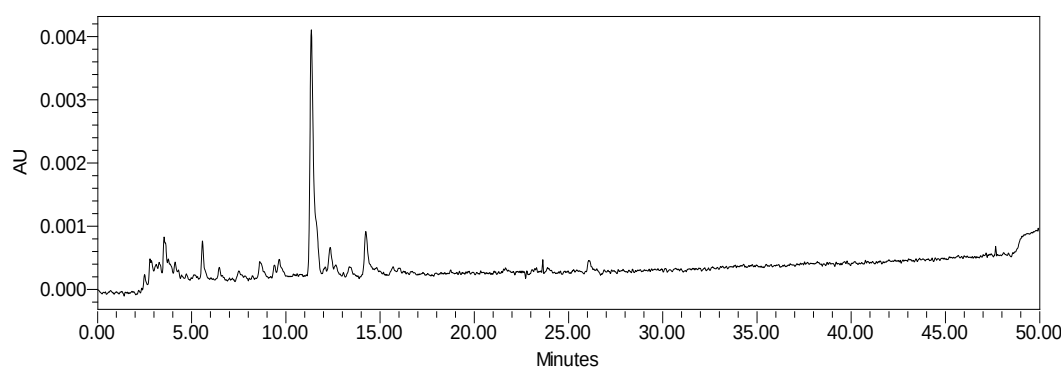


Figure B.46: Representative HPLC chromatograms of apple vinegar PG sample at 312 nm.

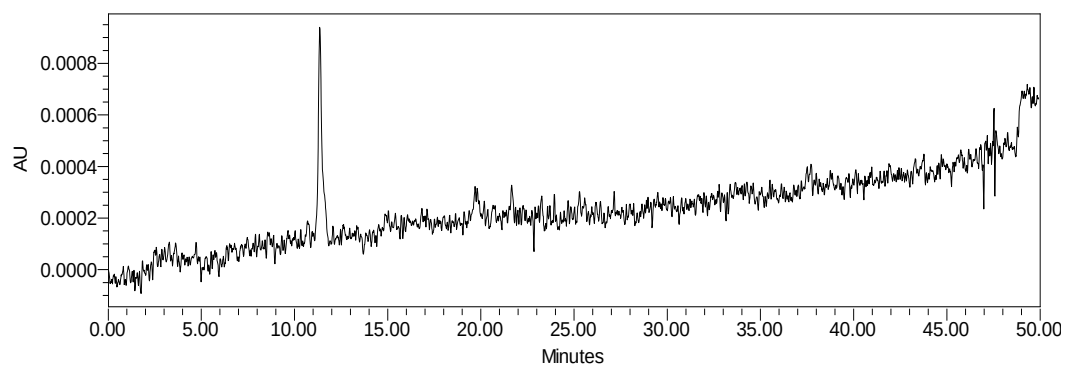


Figure B.47: Representative HPLC chromatograms of apple vinegar PG sample at 360 nm.

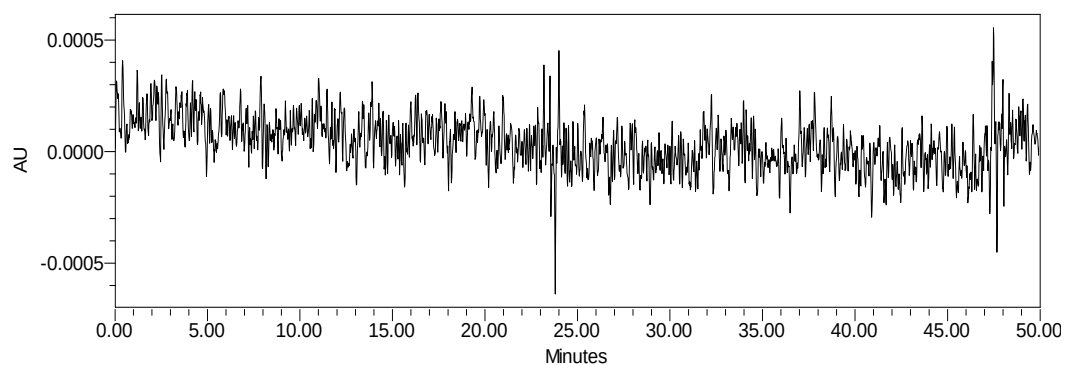


Figure B.48: Representative HPLC chromatograms of apple vinegar PG sample at 520 nm.

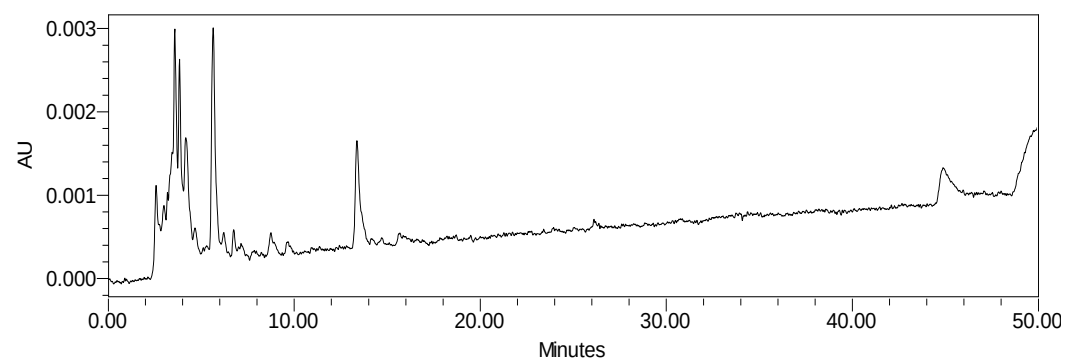


Figure B.49: Representative HPLC chromatograms of apple vinegar IN sample at 280 nm.

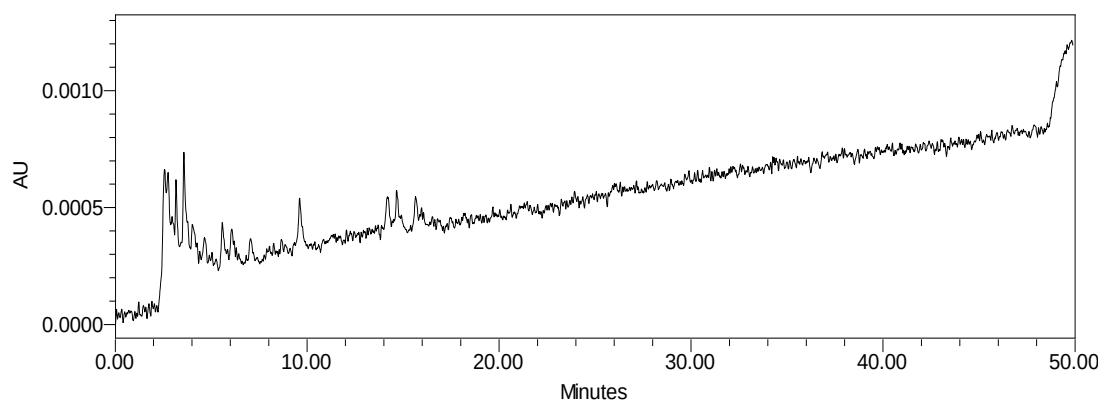


Figure B.50: Representative HPLC chromatograms of apple vinegar IN sample at 312 nm.

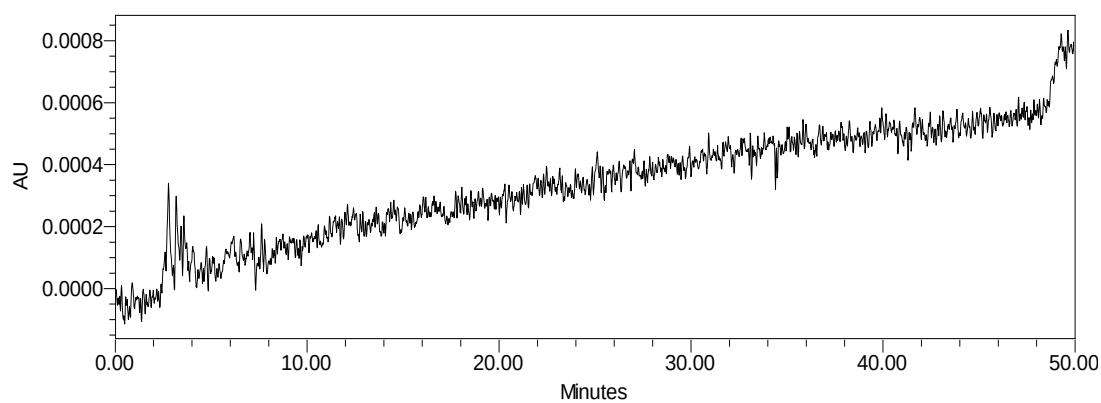


Figure B.51: Representative HPLC chromatograms of apple vinegar IN sample at 360 nm.

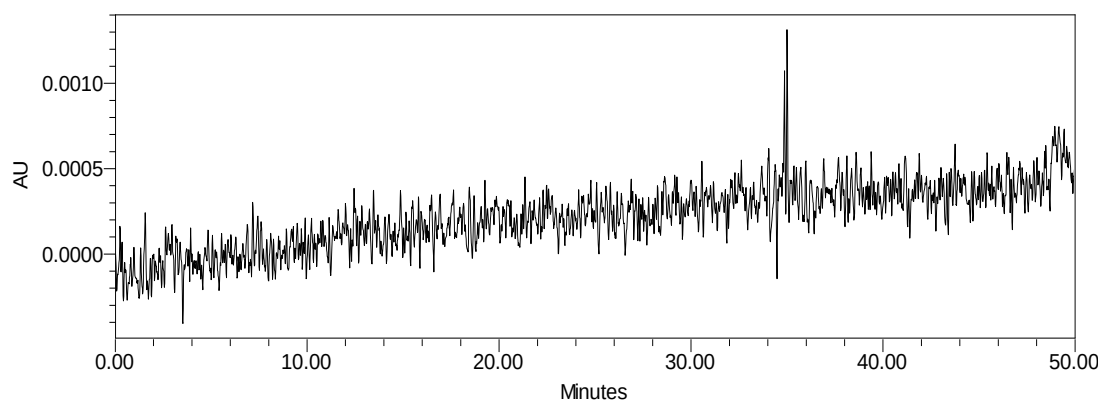


Figure B.52: Representative HPLC chromatograms of apple vinegar IN sample at 520 nm.

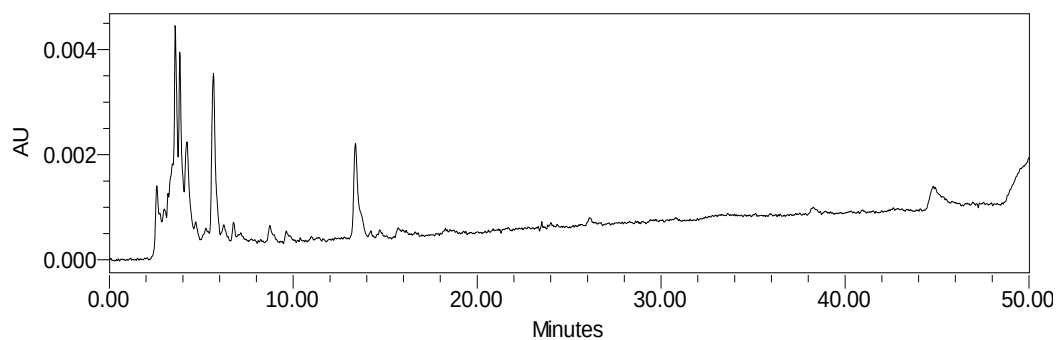


Figure B.53: Representative HPLC chromatograms of apple vinegar OUT sample at 280 nm.

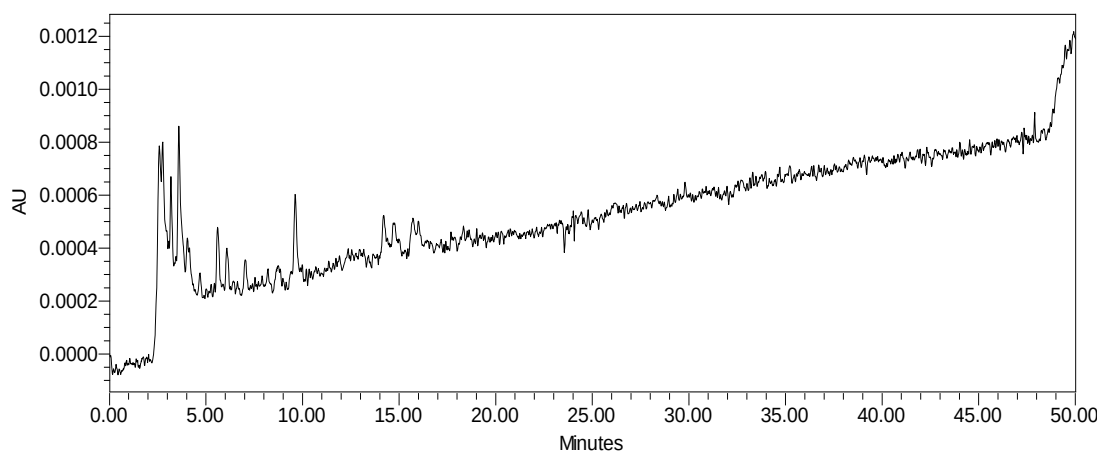


Figure B.54: Representative HPLC chromatograms of apple vinegar OUT sample at 312 nm.

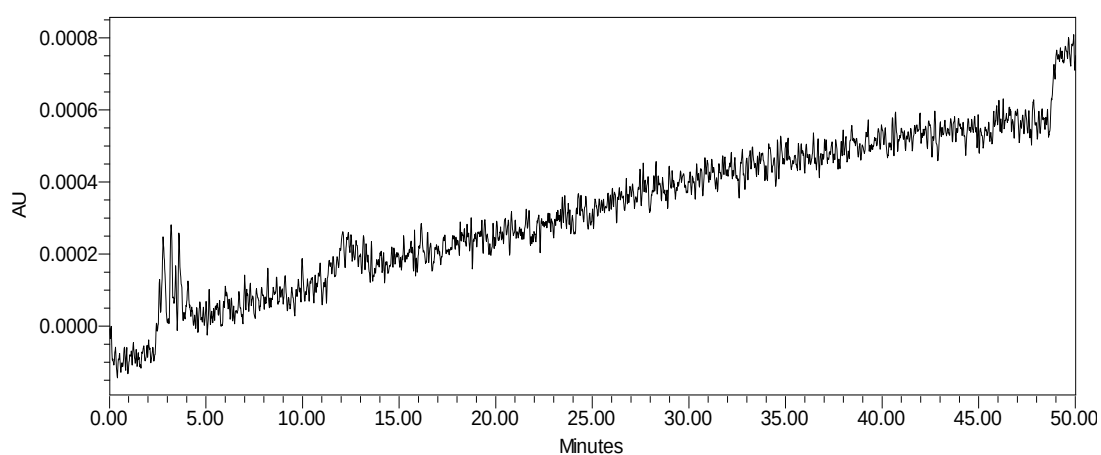


Figure B.55: Representative HPLC chromatograms of apple vinegar OUT sample at 360 nm.

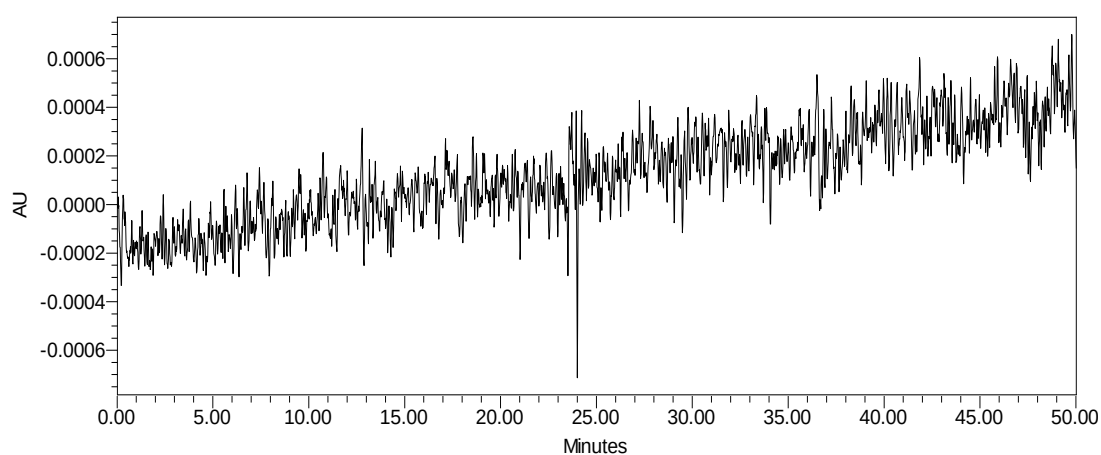


Figure B.56: Representative HPLC chromatograms of apple vinegar OUT sample at 520 nm.

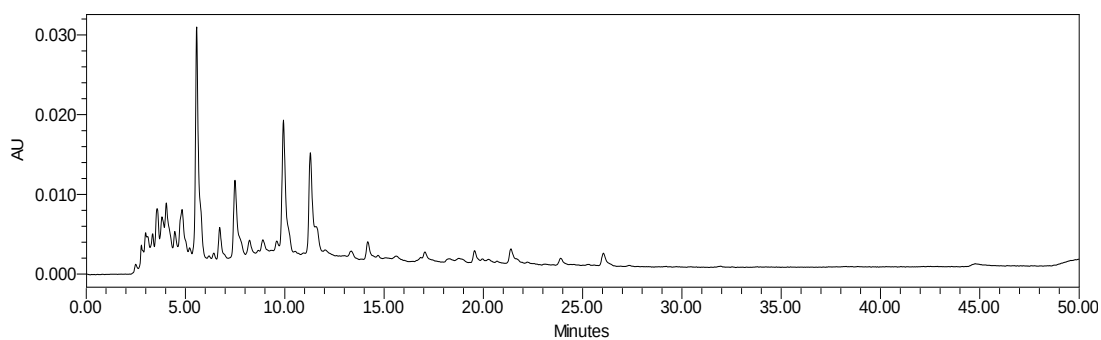


Figure B.57: Representative HPLC chromatograms of pomegranate vinegar IN sample at 280 nm.

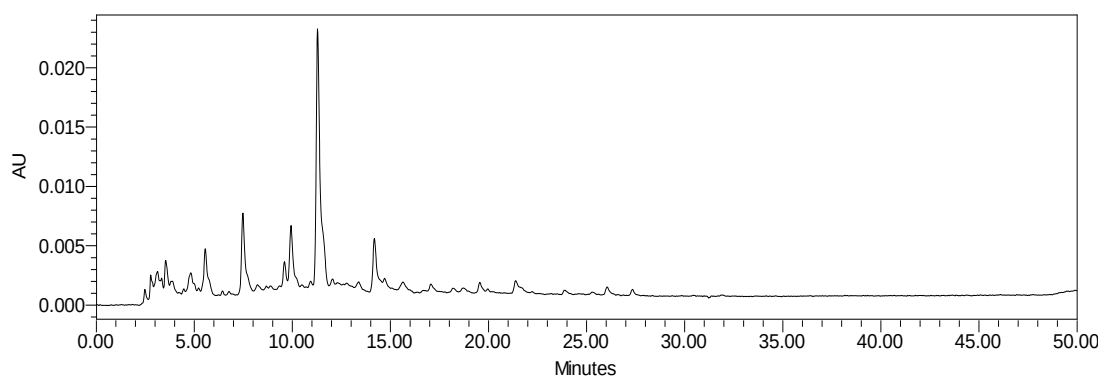


Figure B.58: Representative HPLC chromatograms of pomegranate vinegar IN sample at 312 nm.

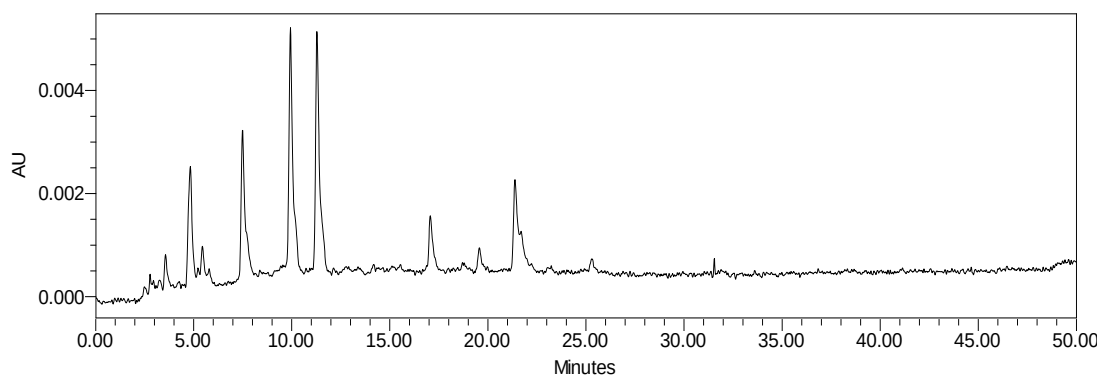


Figure B.59: Representative HPLC chromatograms of pomegranate vinegar IN sample at 360 nm.

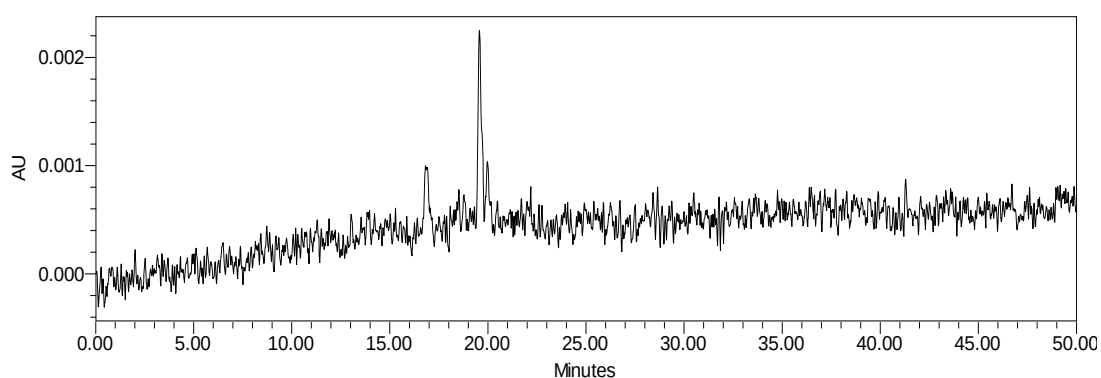


Figure B.60: Representative HPLC chromatograms of pomegranate vinegar IN sample at 520 nm.

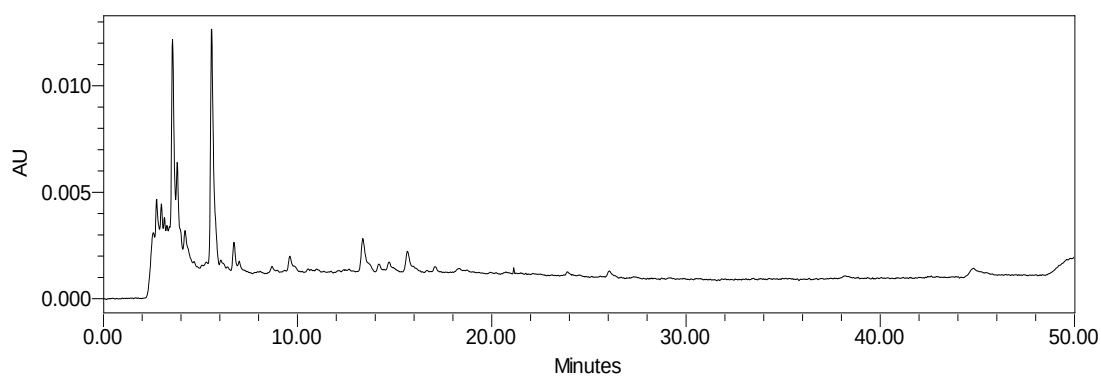


Figure B.61: Representative HPLC chromatograms of pomegranate vinegar OUT sample at 280 nm.

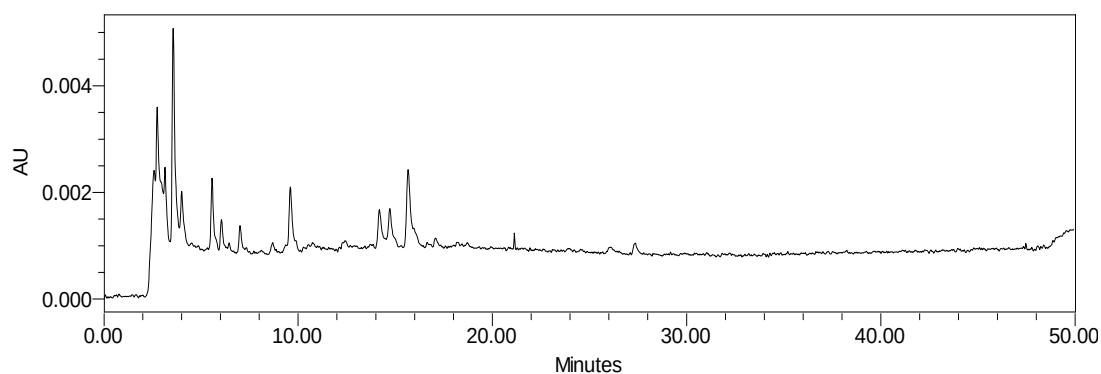


Figure B.62: Representative HPLC chromatograms of pomegranate vinegar OUT sample at 312 nm.

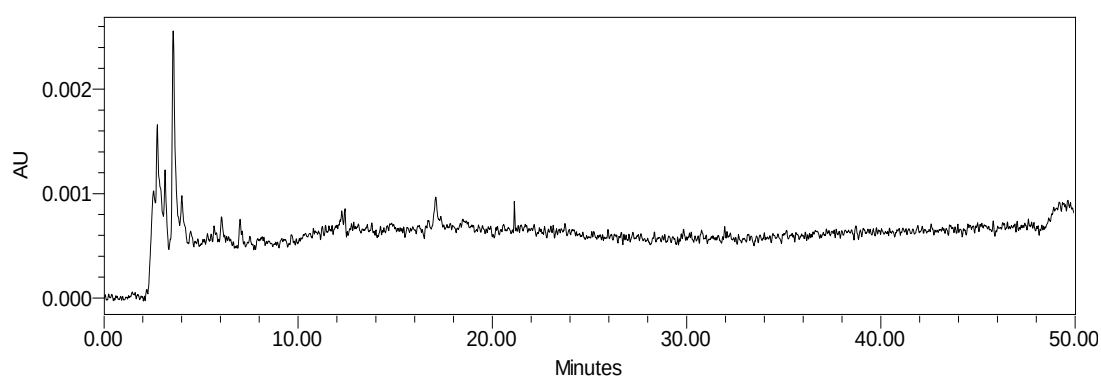


Figure B.63: Representative HPLC chromatograms of pomegranate vinegar OUT sample at 360 nm.

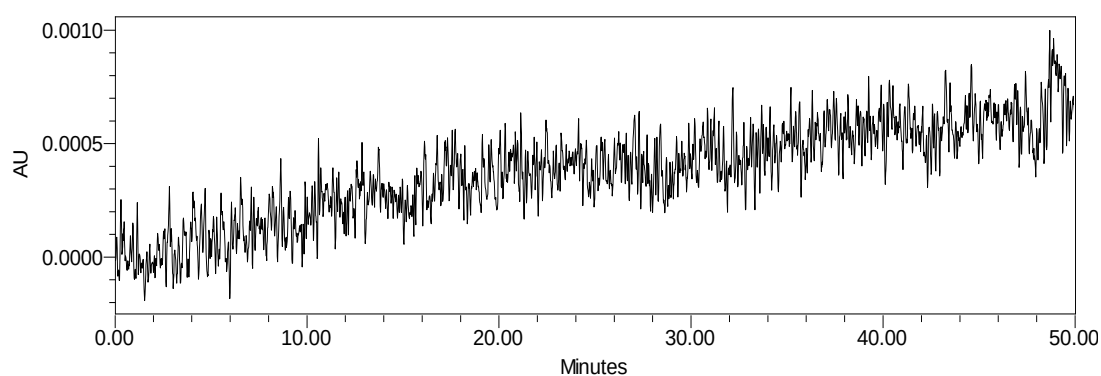


Figure B.64: Representative HPLC chromatograms of pomegranate vinegar OUT sample at 520 nm.

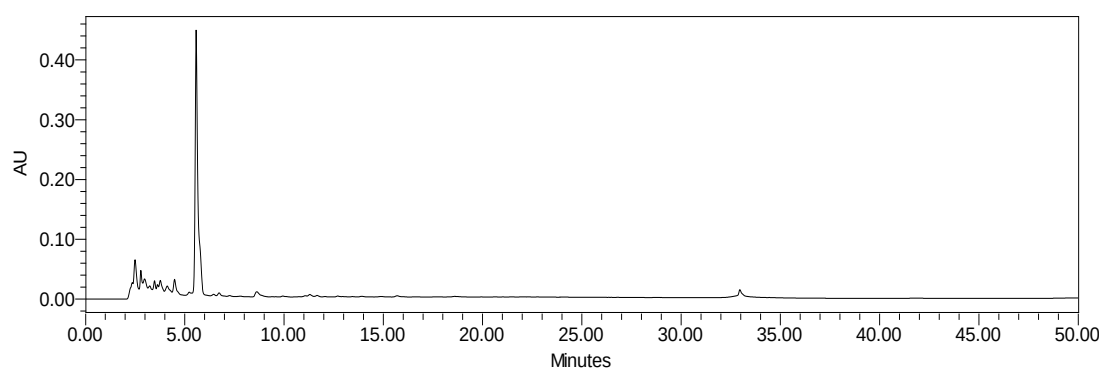


Figure B.65: Representative HPLC chromatograms of balsamic vinegar PG sample at 280 nm.

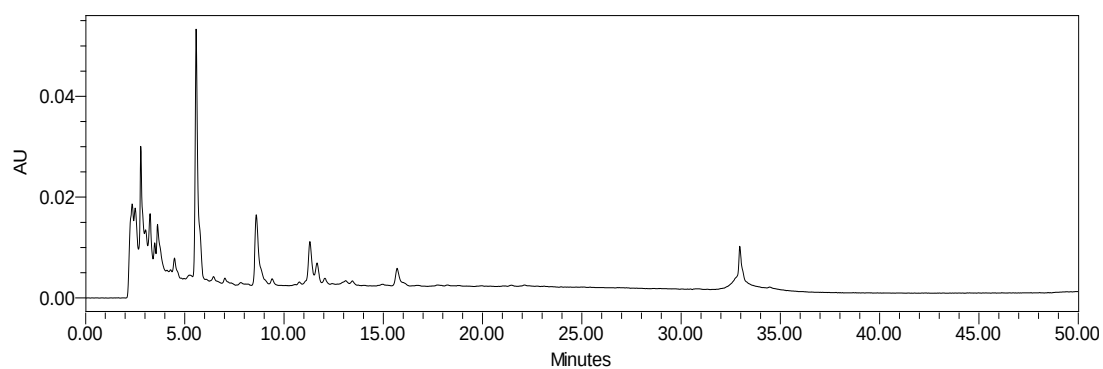


Figure B.66: Representative HPLC chromatograms of balsamic vinegar PG sample at 312 nm.

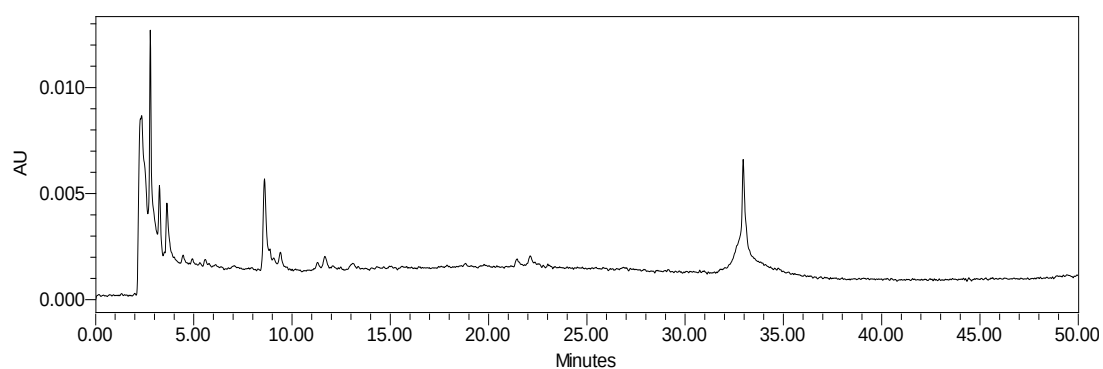


Figure B.67: Representative HPLC chromatograms of balsamic vinegar PG sample at 360 nm.

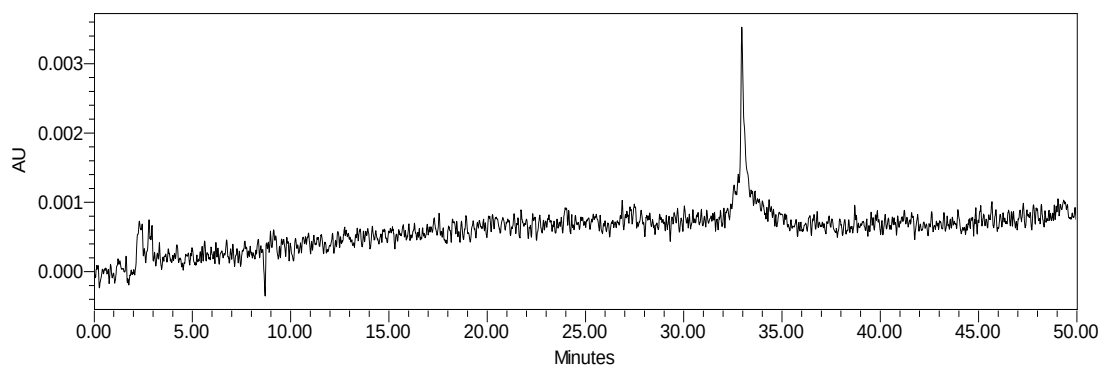


Figure B.68: Representative HPLC chromatograms of balsamic vinegar PG sample at 520 nm.

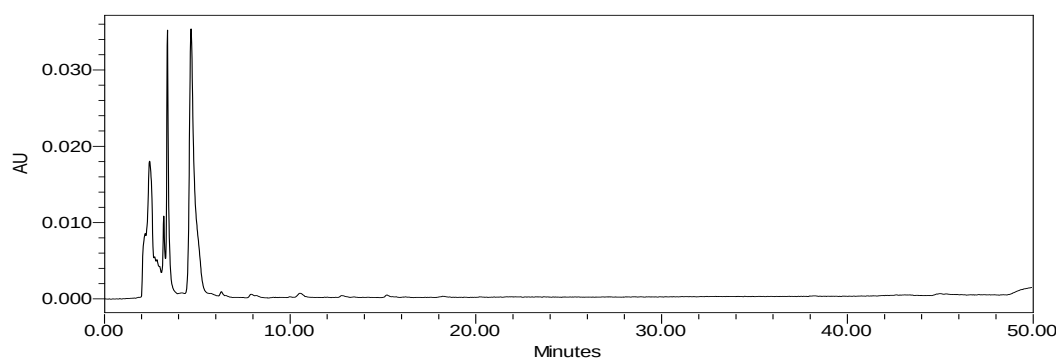


Figure B.69: Representative HPLC chromatograms of balsamic vinegar IN sample at 280 nm.

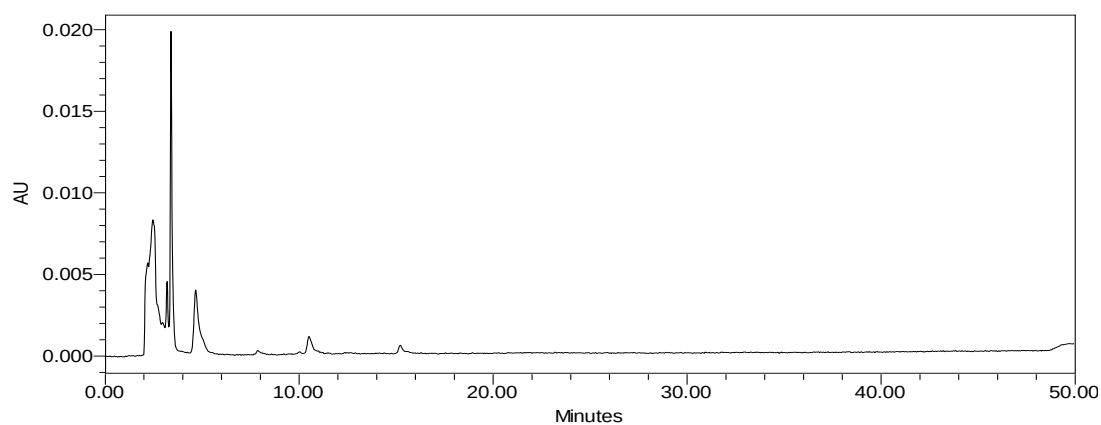


Figure B.70: Representative HPLC chromatograms of balsamic vinegar IN sample at 312 nm.

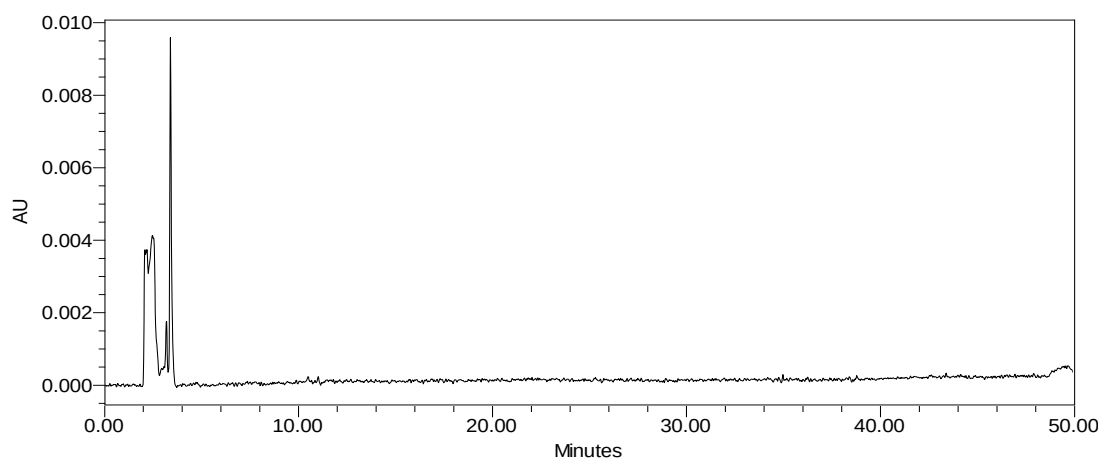


Figure B.71: Representative HPLC chromatograms of balsamic vinegar IN sample at 360 nm.

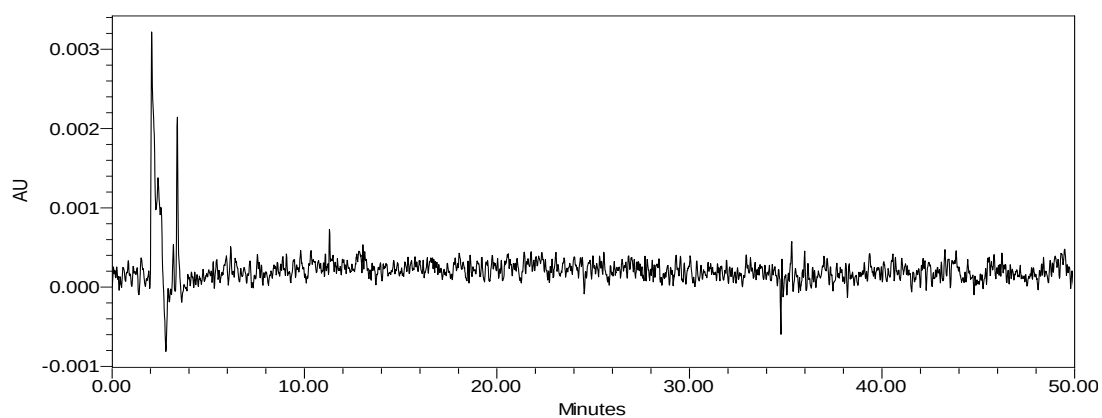


Figure B.72: Representative HPLC chromatograms of balsamic vinegar IN sample at 520 nm.

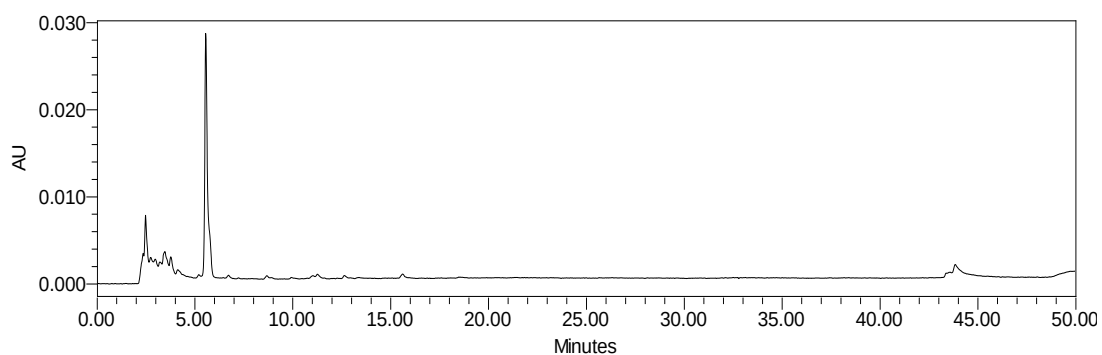


Figure B.73: Representative HPLC chromatograms of balsamic vinegar OUT sample at 280 nm.

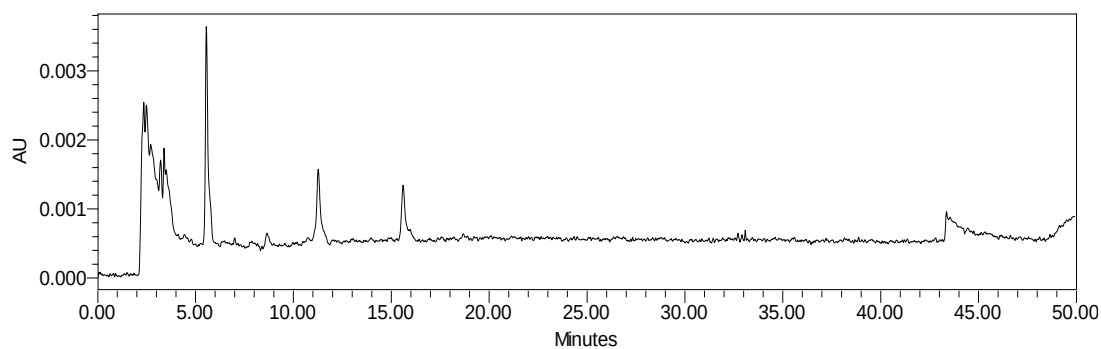


Figure B.74: Representative HPLC chromatograms of balsamic vinegar OUT sample at 312 nm.

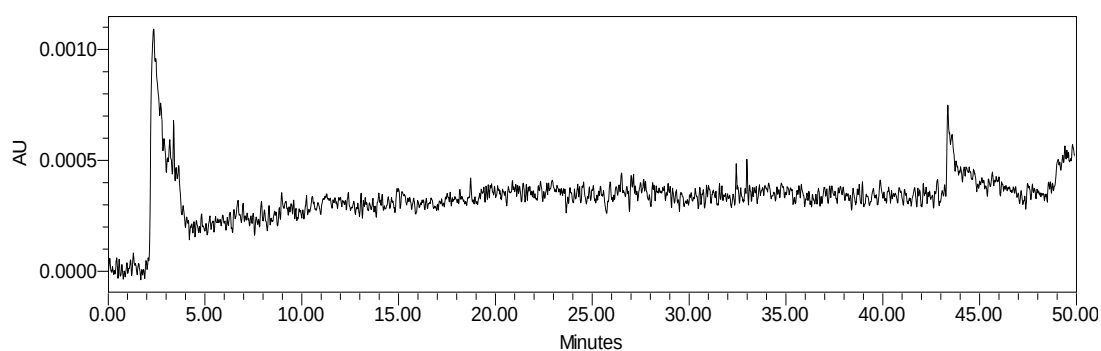


Figure B.75: Representative HPLC chromatograms of balsamic vinegar OUT sample at 360 nm.

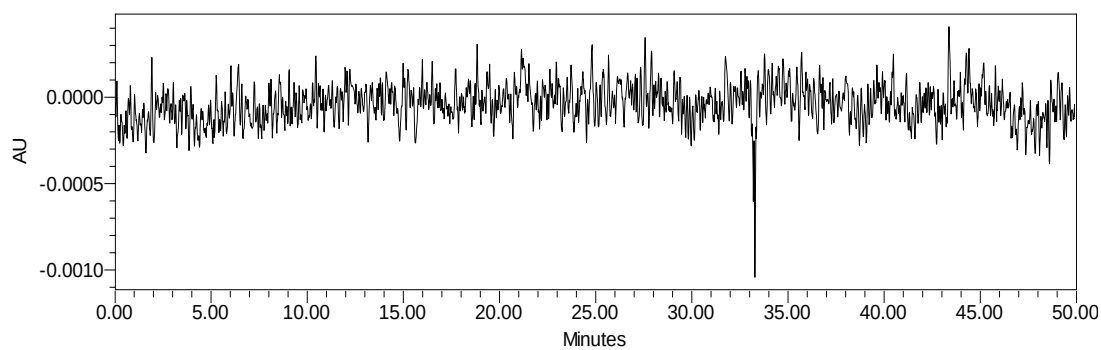


Figure B.76: Representative HPLC chromatograms of balsamic vinegar OUT sample at 520 nm.

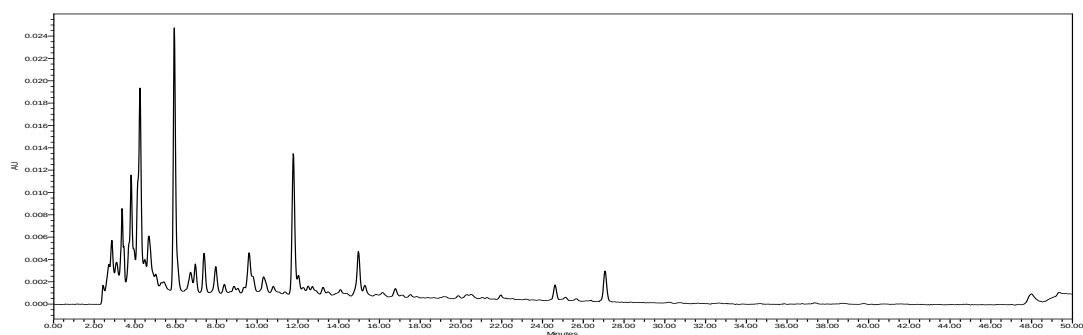


Figure B.77: Representative HPLC chromatograms of apple vinegar I sample at 280 nm.

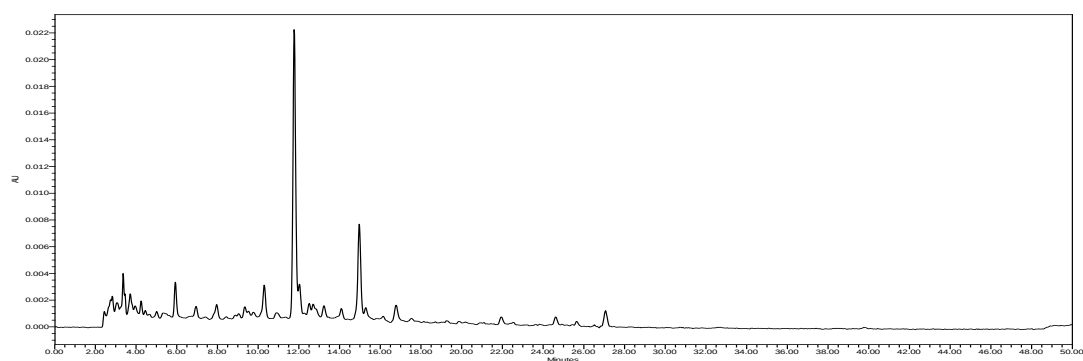


Figure B.78: Representative HPLC chromatograms of apple vinegar I sample at 312 nm.

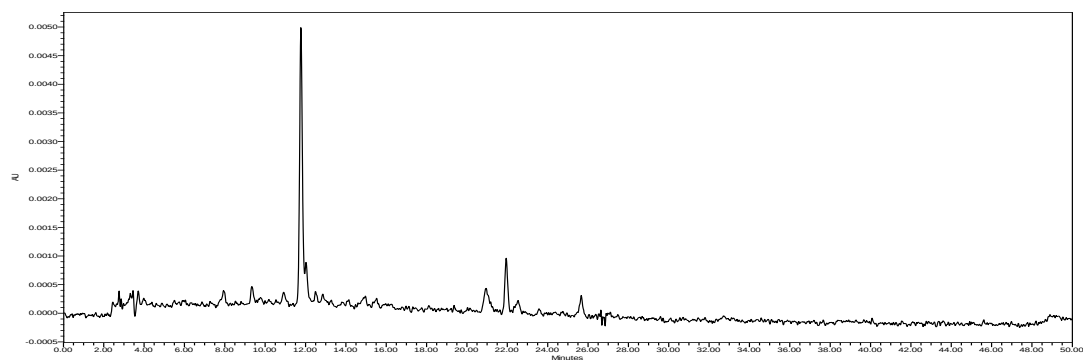


Figure B.79: Representative HPLC chromatograms of apple vinegar I sample at 360 nm.

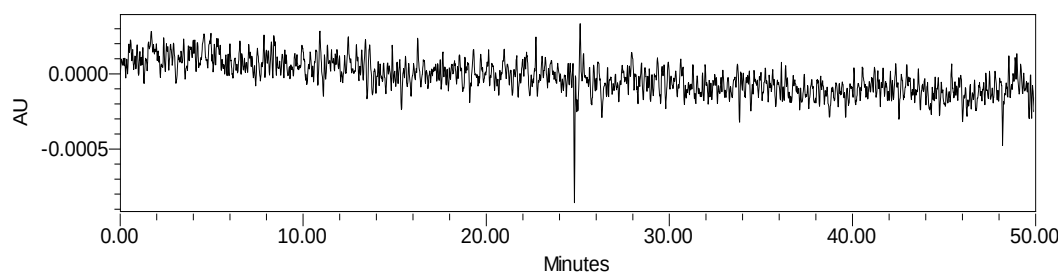


Figure B.80: Representative HPLC chromatograms of apple vinegar I sample at 520 nm.

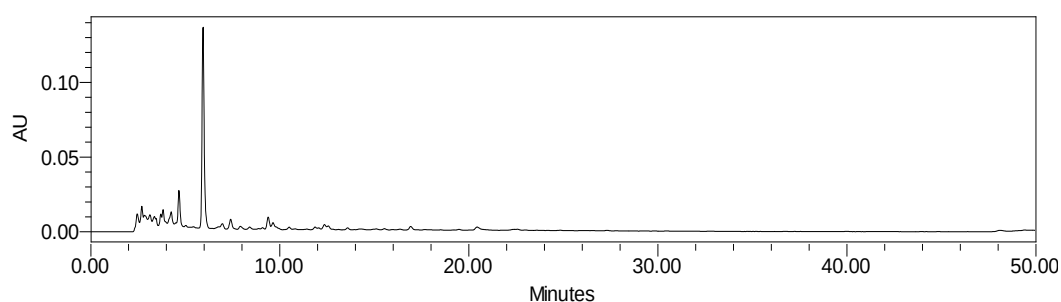


Figure B.81: Representative HPLC chromatograms of grape vinegar I sample at 280 nm.

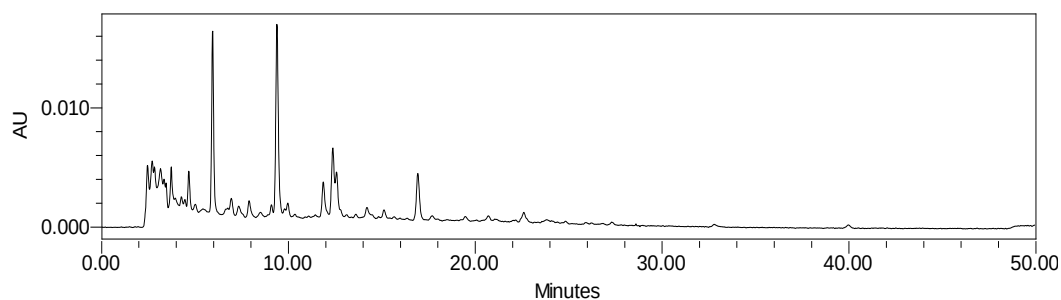


Figure B.82: Representative HPLC chromatograms of grape vinegar I sample at 312 nm.

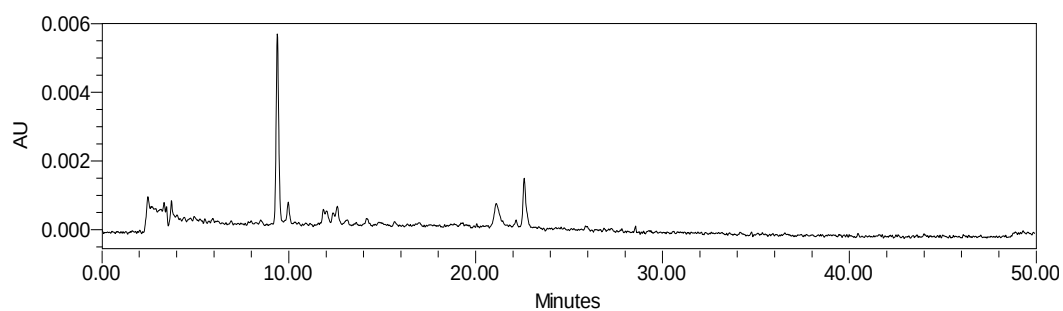


Figure B.83: Representative HPLC chromatograms of grape vinegar I sample at 360 nm.

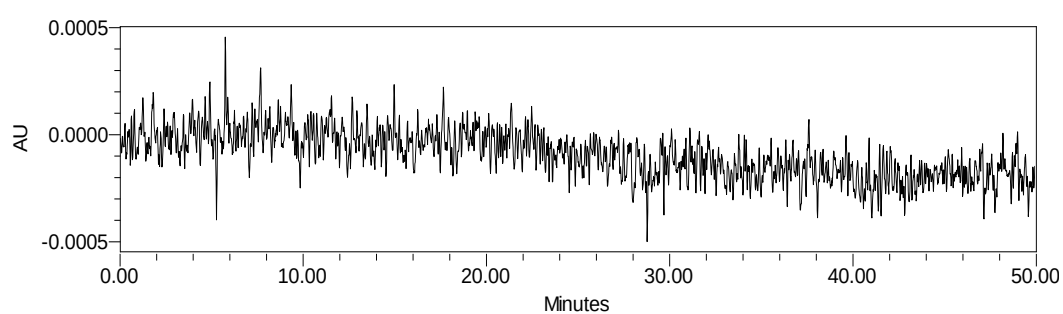


Figure B.84: Representative HPLC chromatograms of grape vinegar I sample at 520 nm.

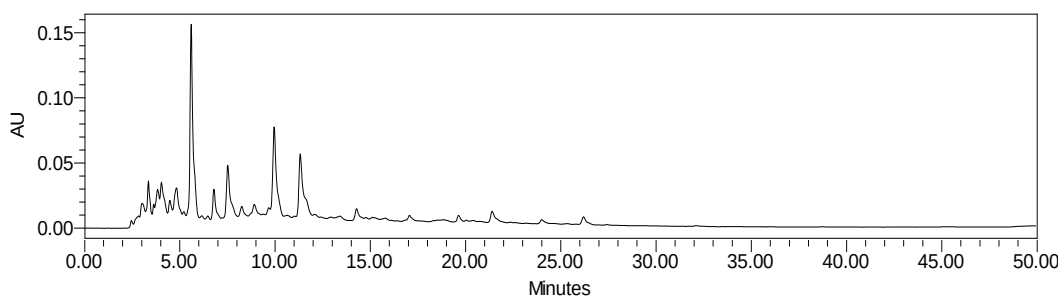


Figure B.85: Representative HPLC chromatograms of pomegranate vinegar I sample at 280 nm.

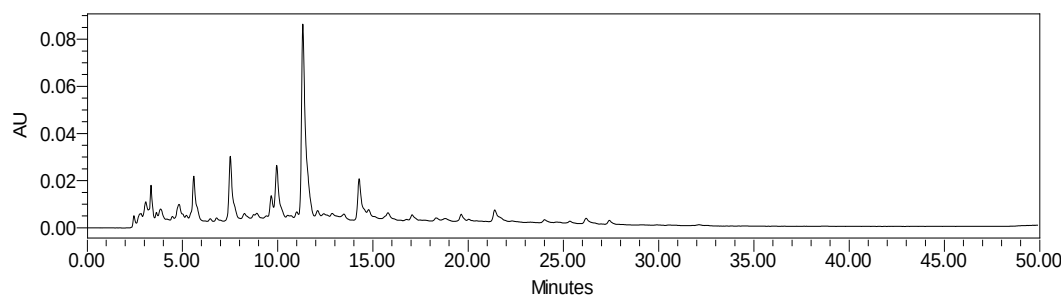


Figure B.86: Representative HPLC chromatograms of pomegranate vinegar I sample at 312 nm.

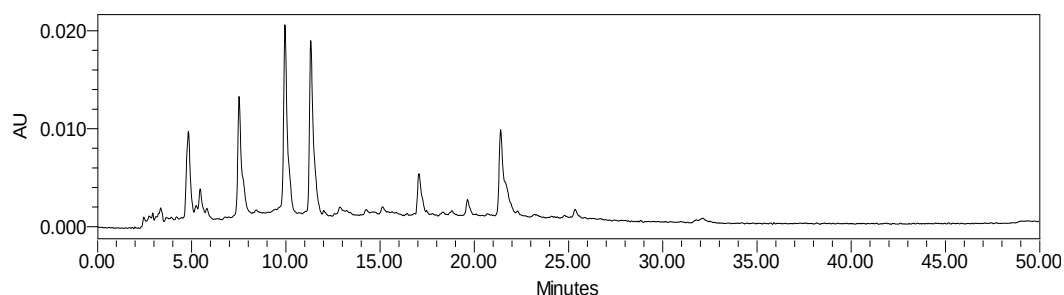


Figure B.87: Representative HPLC chromatograms of pomegranate vinegar I sample at 360 nm.

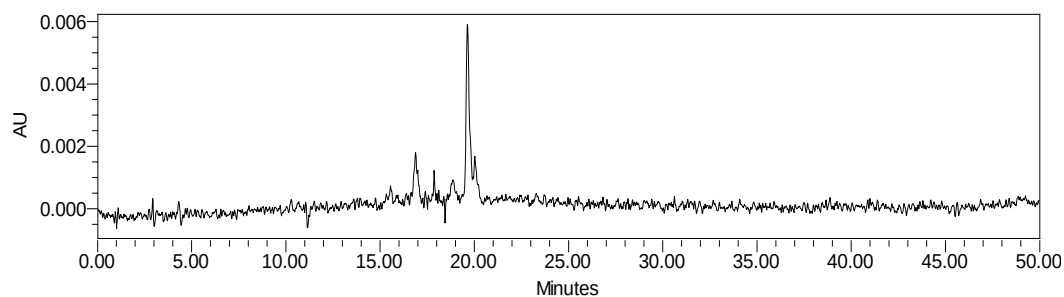


Figure B.88: Representative HPLC chromatograms of pomegranate vinegar I sample at 520 nm.

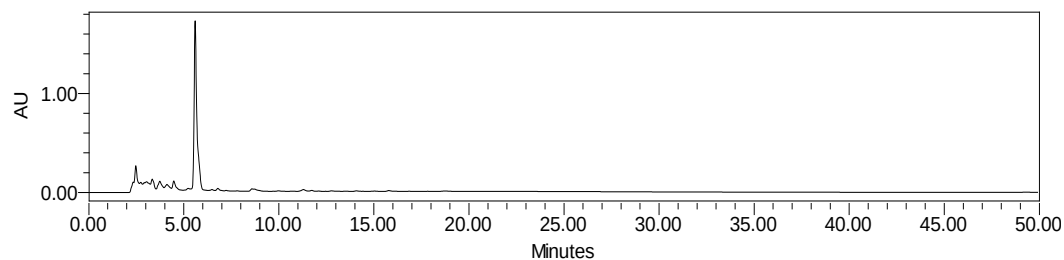


Figure B.89: Representative HPLC chromatograms of balsamic vinegar sample at 280 nm.

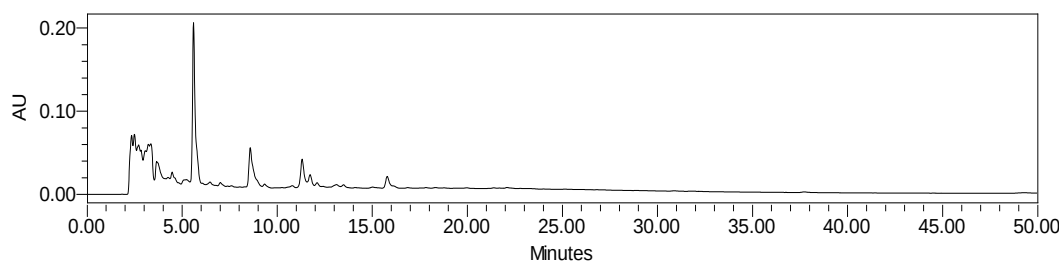


Figure B.90: Representative HPLC chromatograms of balsamic vinegar sample at 312 nm.

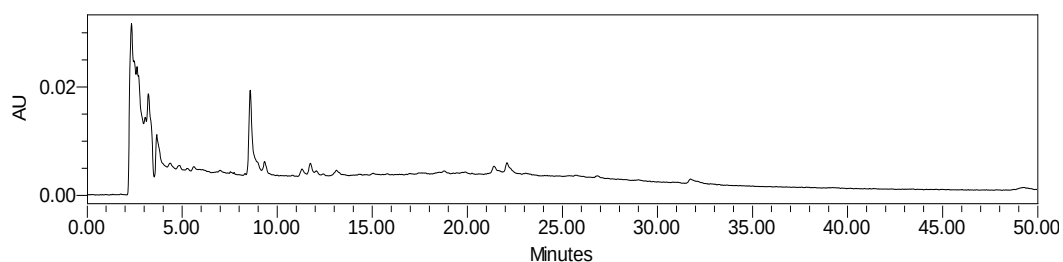


Figure B.91: Representative HPLC chromatograms of balsamic vinegar sample at 360 nm.

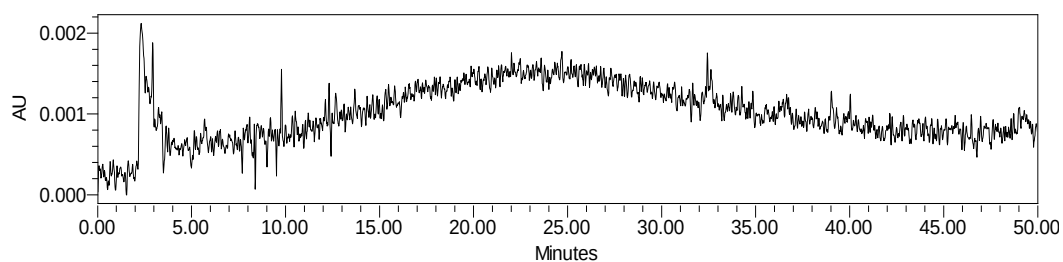


Figure B.92: Representative HPLC chromatograms of balsamic vinegar sample at 520 nm.

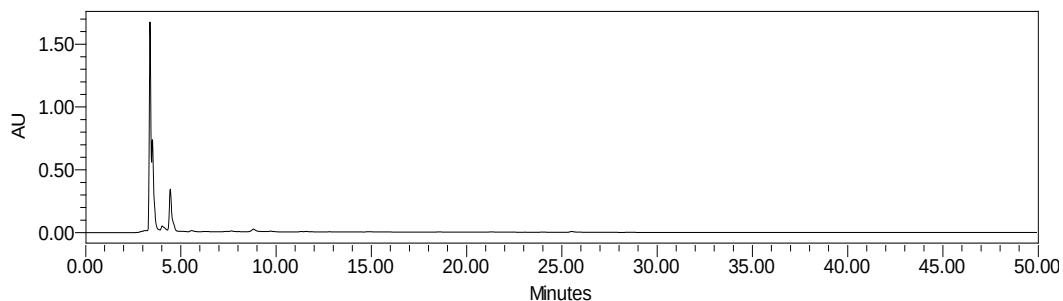


Figure B.93: Representative HPLC chromatograms of blueberry vinegar sample at 280 nm.

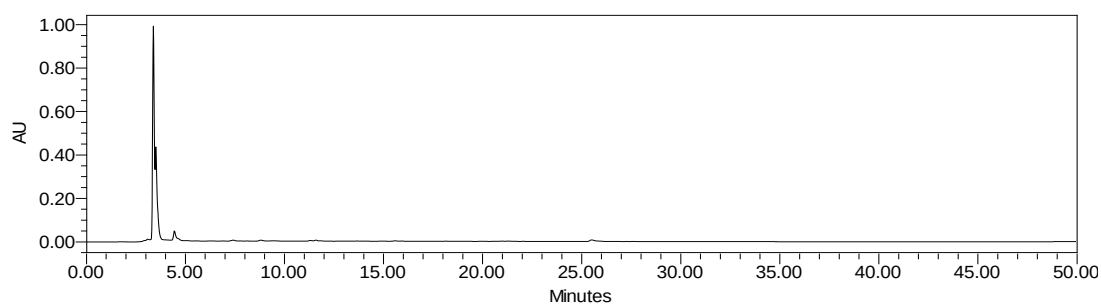


Figure B.94: Representative HPLC chromatograms of blueberry vinegar sample at 312 nm.

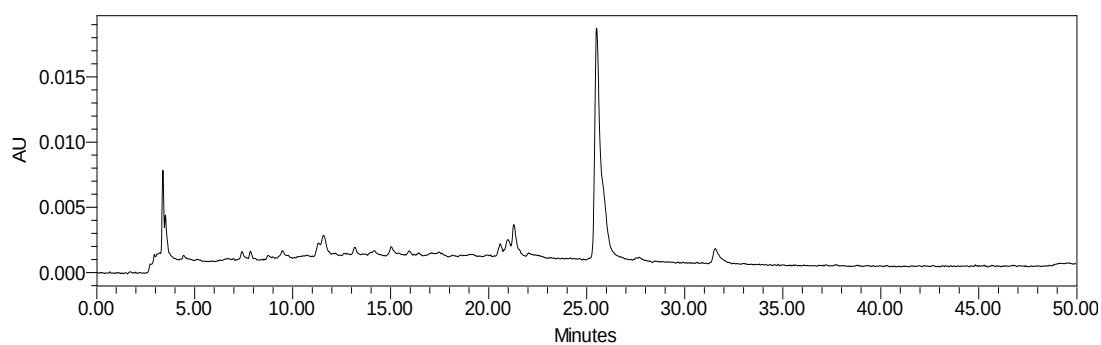


Figure B.95: Representative HPLC chromatograms of blueberry vinegar sample at 360 nm.

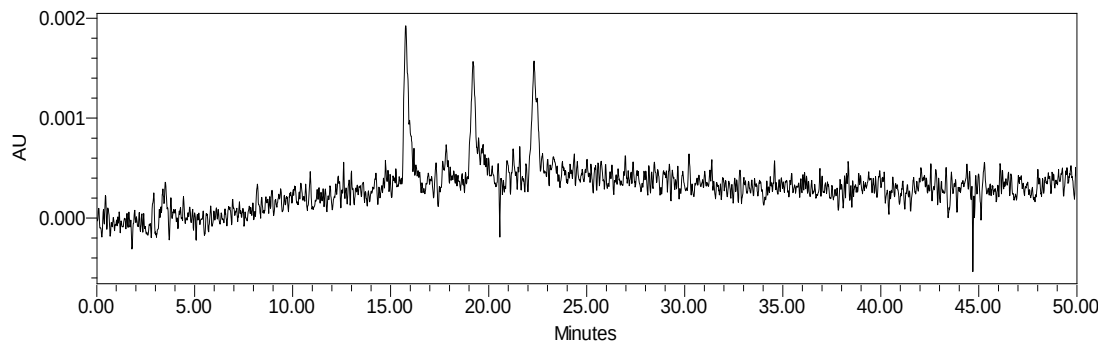


Figure B.96: Representative HPLC chromatograms of blueberry vinegar sample at 520 nm.

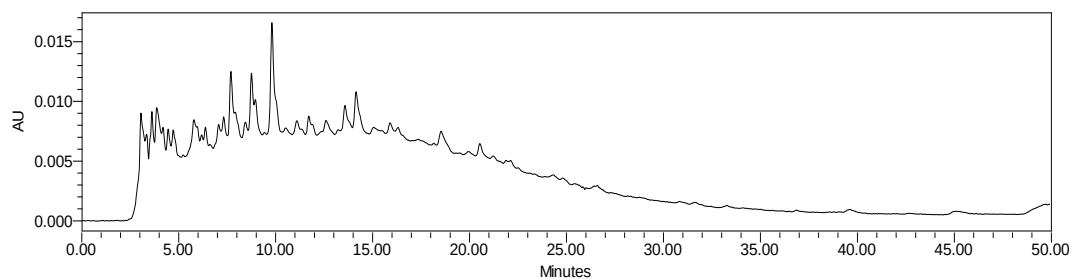


Figure B.97: Representative HPLC chromatograms of rosehip vinegar sample at 280 nm.

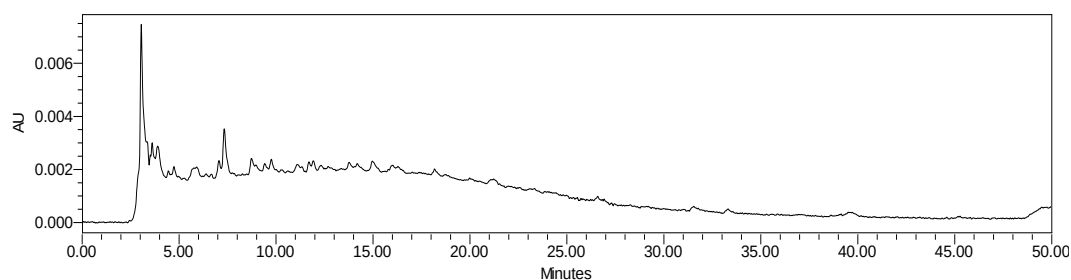


Figure B.98: Representative HPLC chromatograms of rosehip vinegar sample at 312 nm.

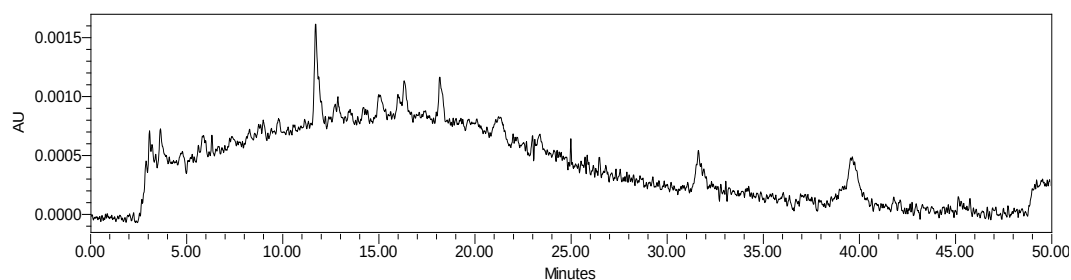


Figure B.99: Representative HPLC chromatograms of rosehip vinegar sample at 360 nm.

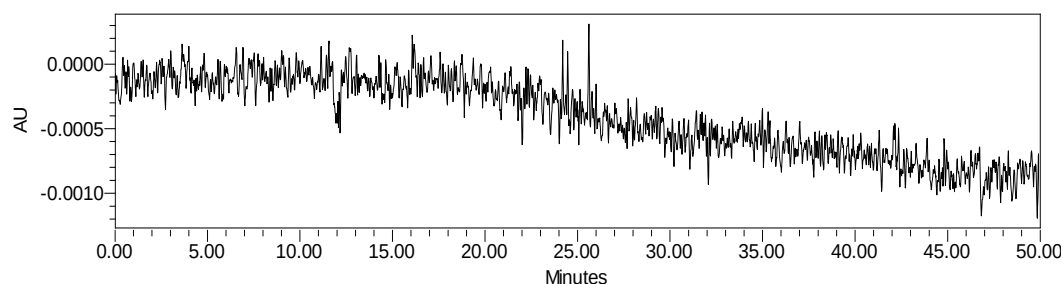


Figure B.100: Representative HPLC chromatograms of rosehip vinegar sample at 520 nm.

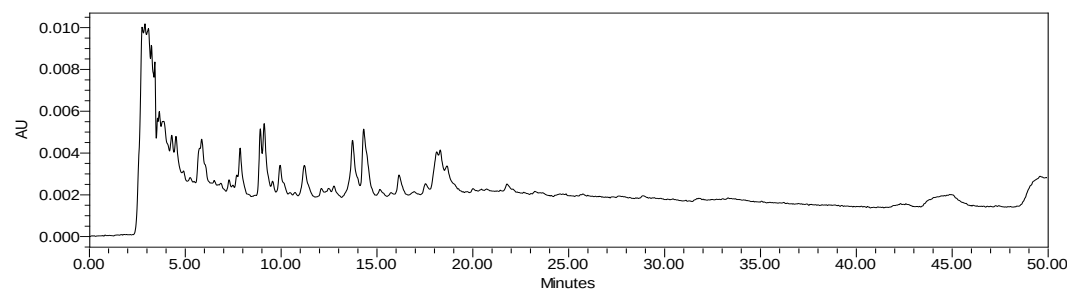


Figure B.101: Representative HPLC chromatograms of pomegranate vinegar II sample at 280 nm.

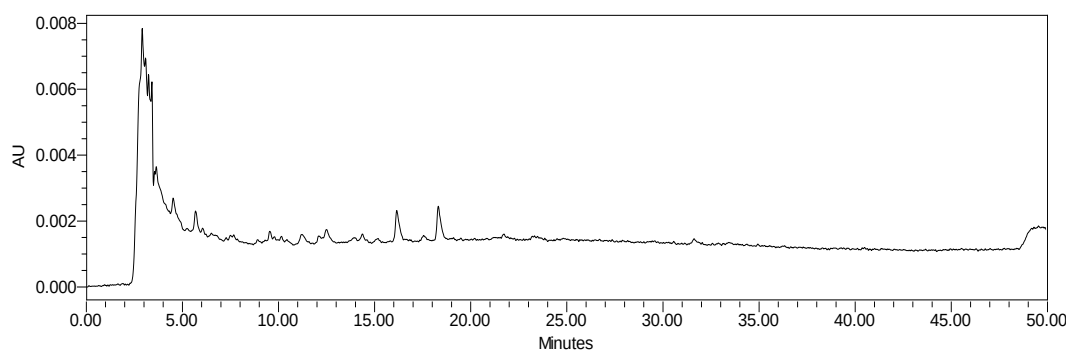


Figure B.102: Representative HPLC chromatograms of pomegranate vinegar II sample at 312 nm.

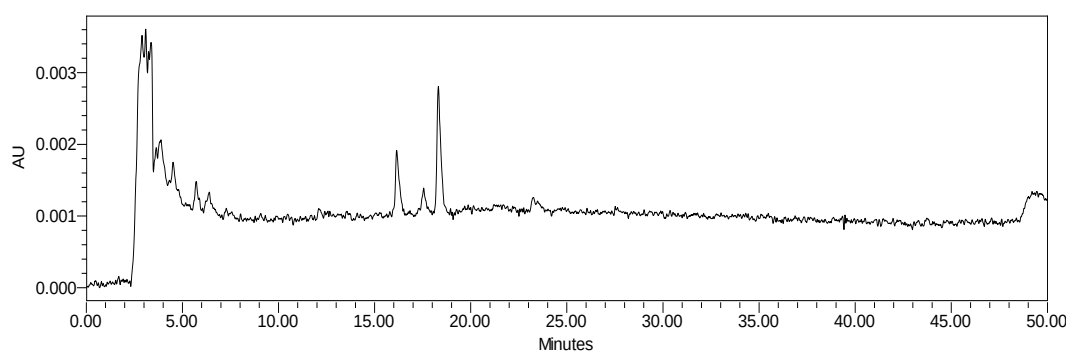


Figure B.103: Representative HPLC chromatograms of pomegranate vinegar II sample at 360 nm.

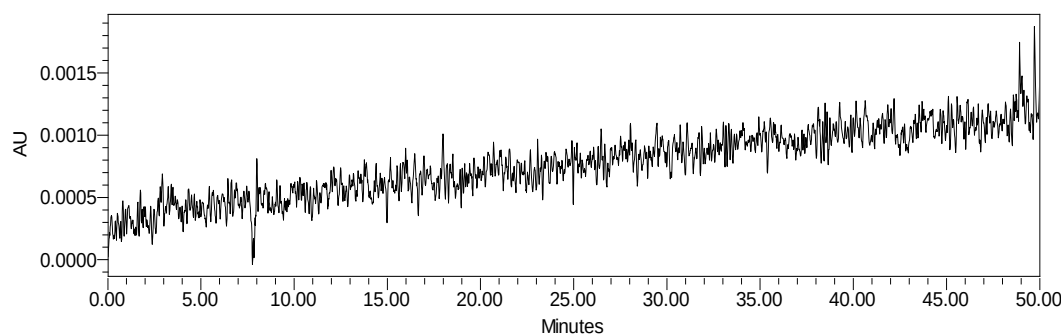


Figure B.104: Representative HPLC chromatograms of pomegranate vinegar II sample at 520 nm.

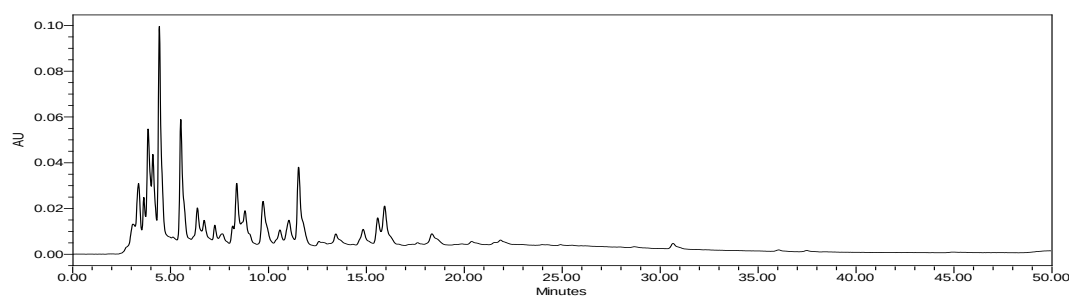


Figure B.105: Representative HPLC chromatograms of grape vinegar II sample at 280 nm.

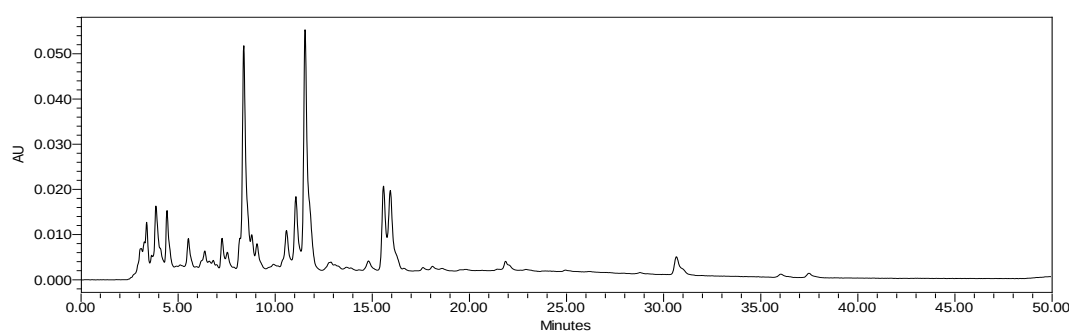


Figure B.106: Representative HPLC chromatograms of grape vinegar II sample at 312 nm.

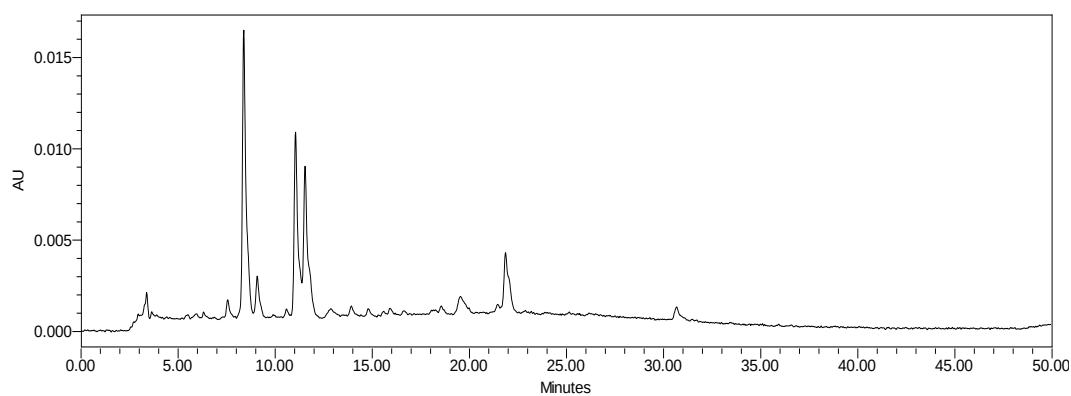


Figure B.107: Representative HPLC chromatograms of grape vinegar II sample at 360 nm.

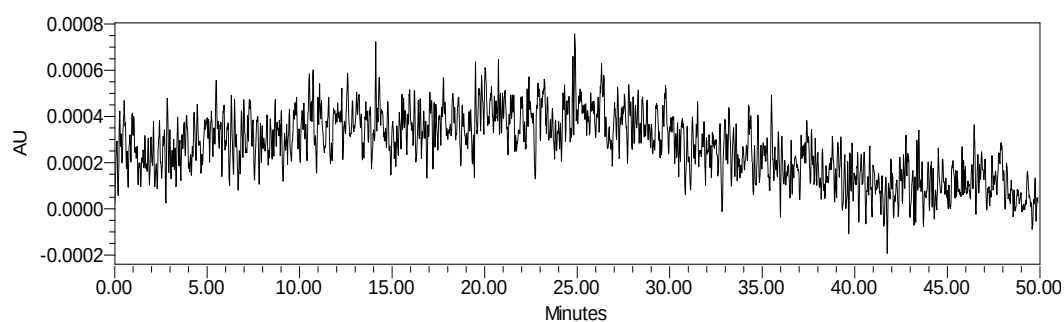


Figure B.108: Representative HPLC chromatograms of grape vinegar II sample at 520 nm.

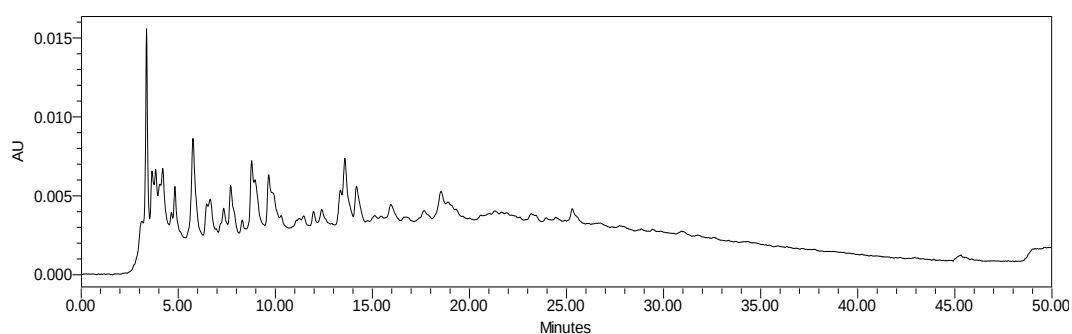


Figure B.109: Representative HPLC chromatograms of gilaburu vinegar sample at 280 nm.

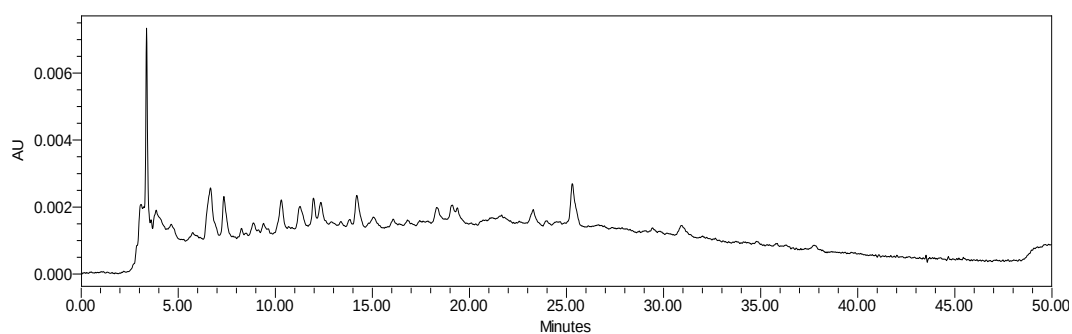


Figure B.110: Representative HPLC chromatograms of gilaburu vinegar sample at 312 nm.

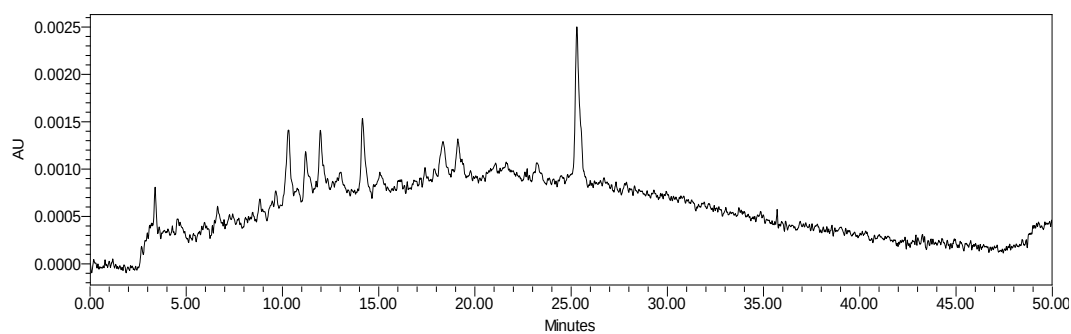


Figure B.111: Representative HPLC chromatograms of gilaburu vinegar sample at 360 nm.

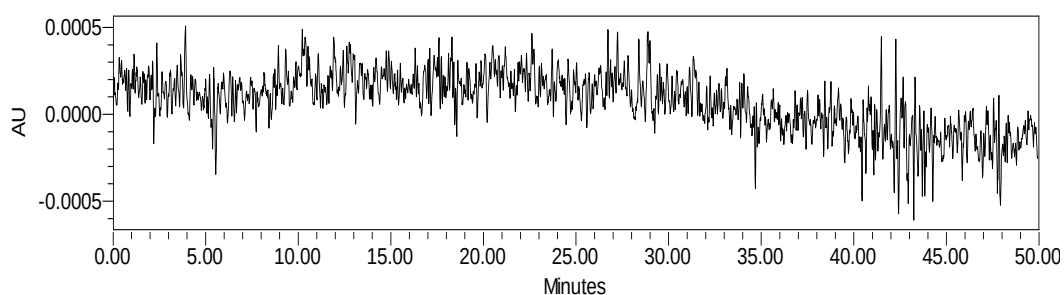


Figure B.112: Representative HPLC chromatograms of gilaburu vinegar sample at 520 nm.

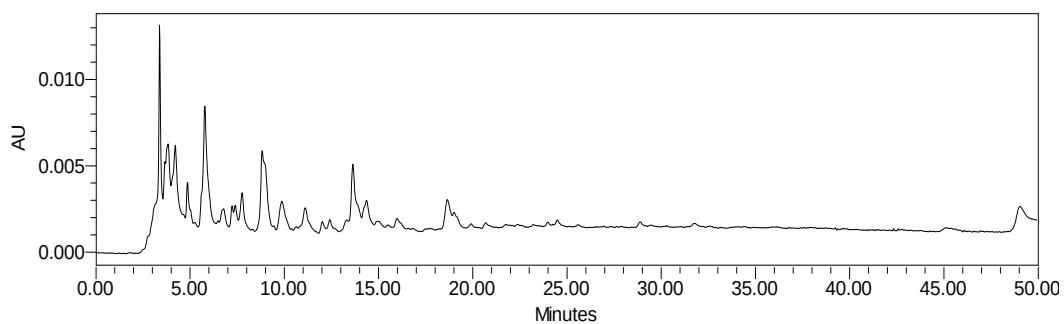


Figure B.113: Representative HPLC chromatograms of apple vinegar II sample at 280 nm.

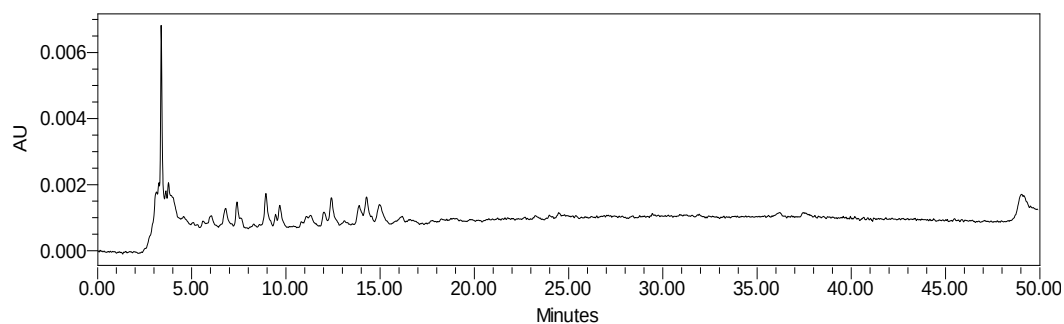


Figure B.114: Representative HPLC chromatograms of apple vinegar II sample at 312 nm.

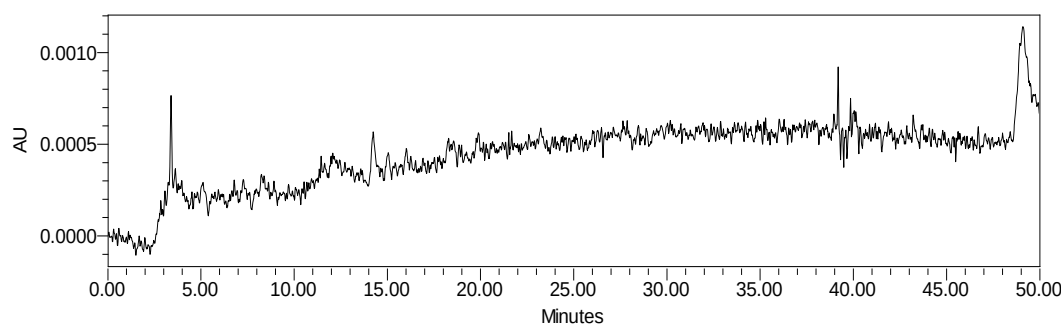


Figure B.115: Representative HPLC chromatograms of apple vinegar II sample at 360 nm.

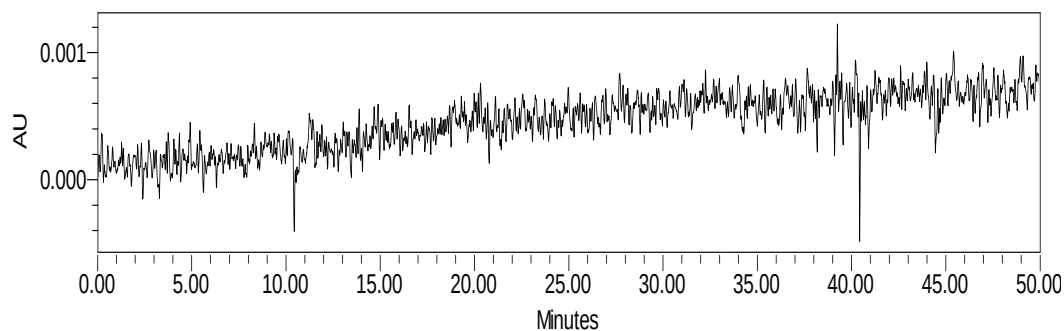


Figure B.116: Representative HPLC chromatograms of apple vinegar II sample at 520 nm.

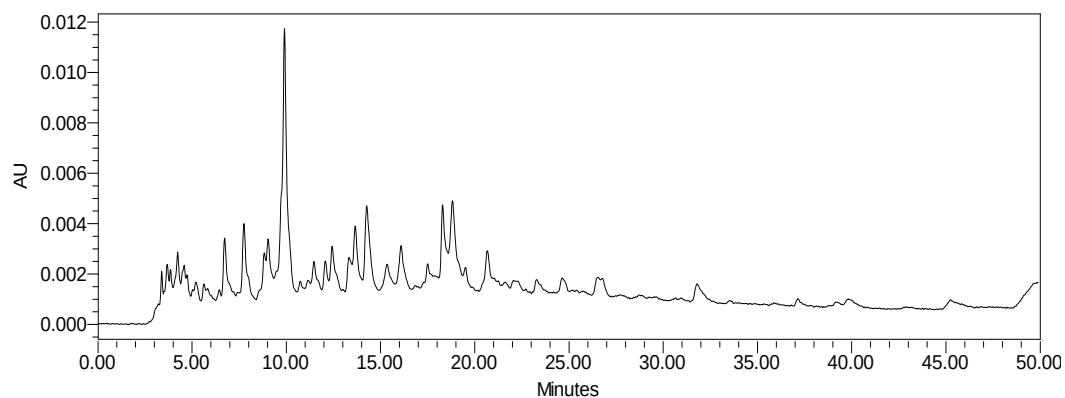


Figure B.117: Representative HPLC chromatograms of lemon vinegar sample at 280 nm.

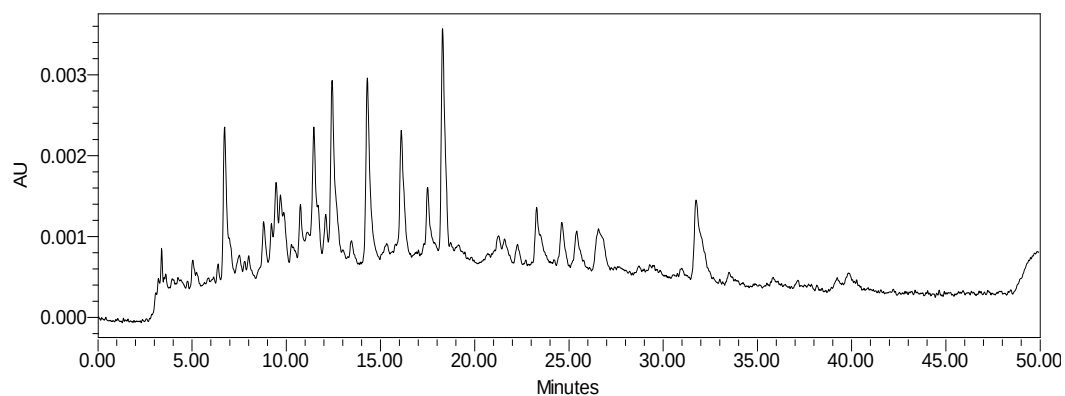


Figure B.118: Representative HPLC chromatograms of lemon vinegar sample at 312 nm.

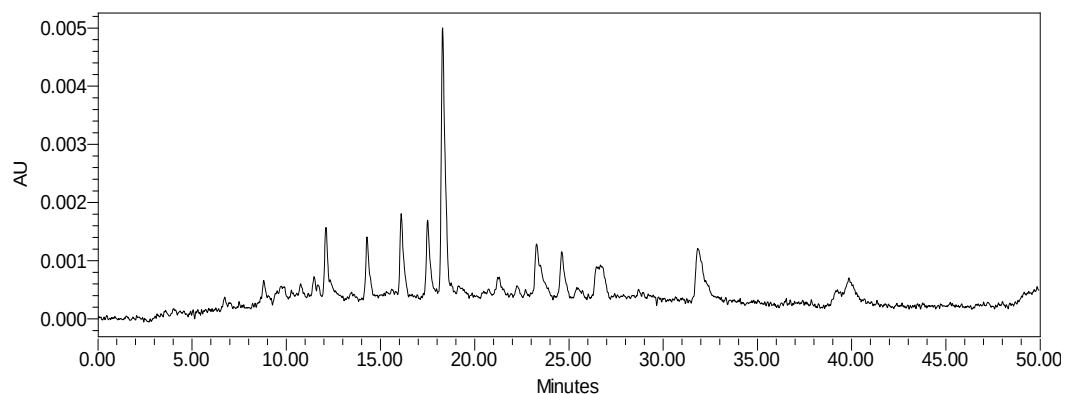


Figure B.119: Representative HPLC chromatograms of lemon vinegar sample at 360 nm.

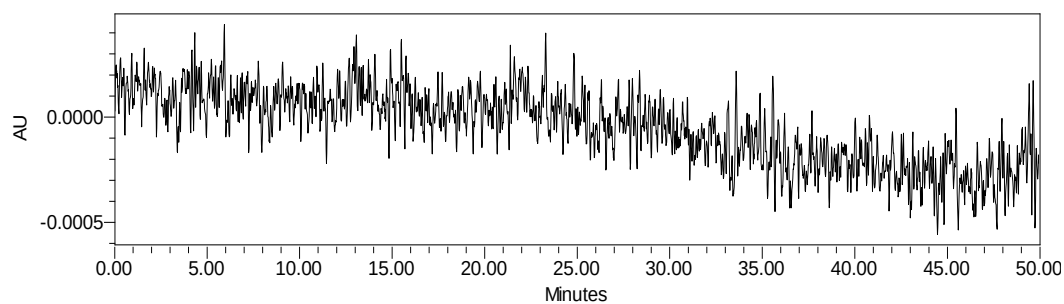


Figure B.120: Representative HPLC chromatograms of lemon vinegar sample at 520 nm.

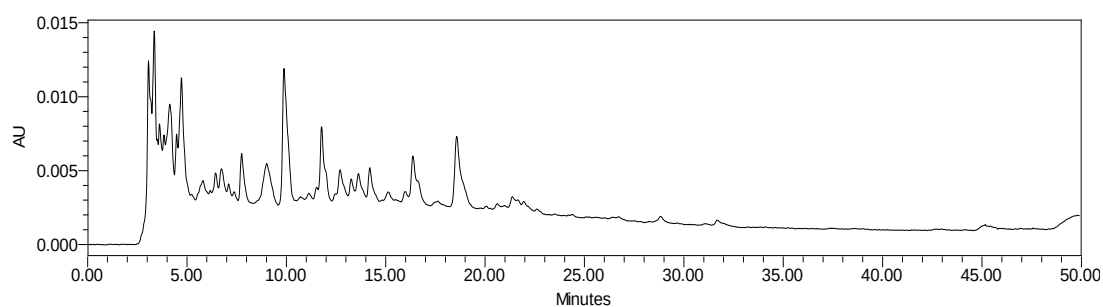


Figure B.121: Representative HPLC chromatograms of blackberry vinegar sample at 280 nm.

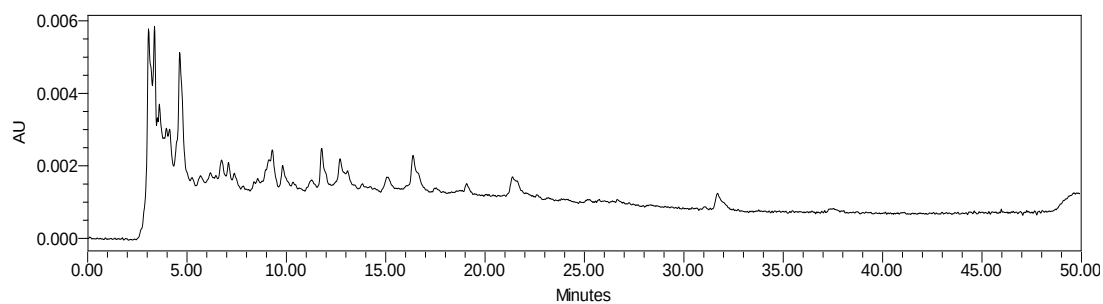


Figure B.122: Representative HPLC chromatograms of blackberry vinegar sample at 312 nm.

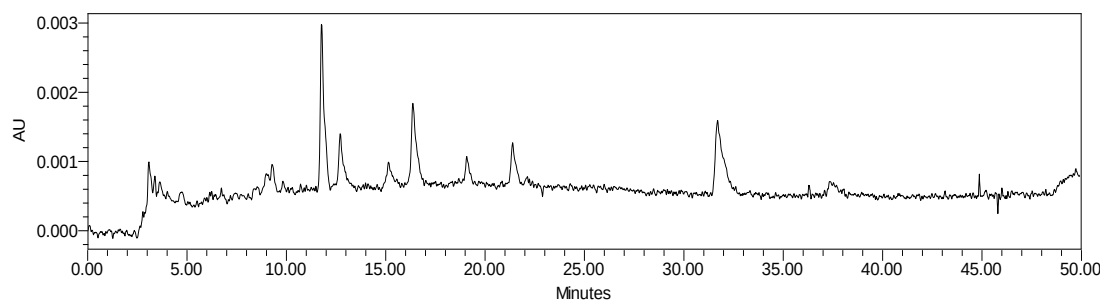


Figure B.123: Representative HPLC chromatograms of blackberry vinegar sample at 360 nm.

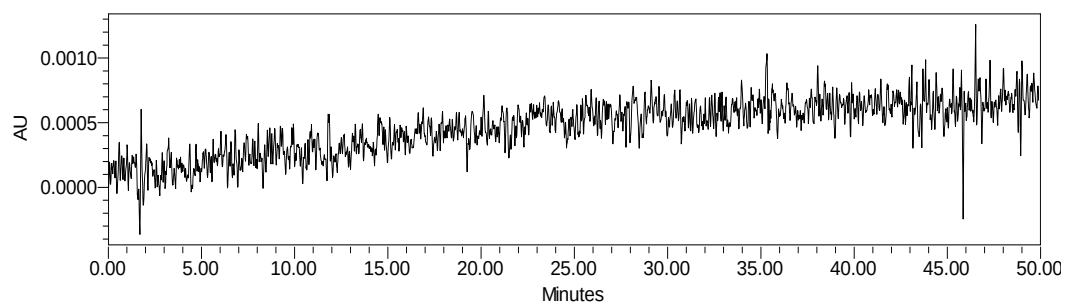


Figure B.124: Representative HPLC chromatograms of blackberry vinegar sample at 520 nm.

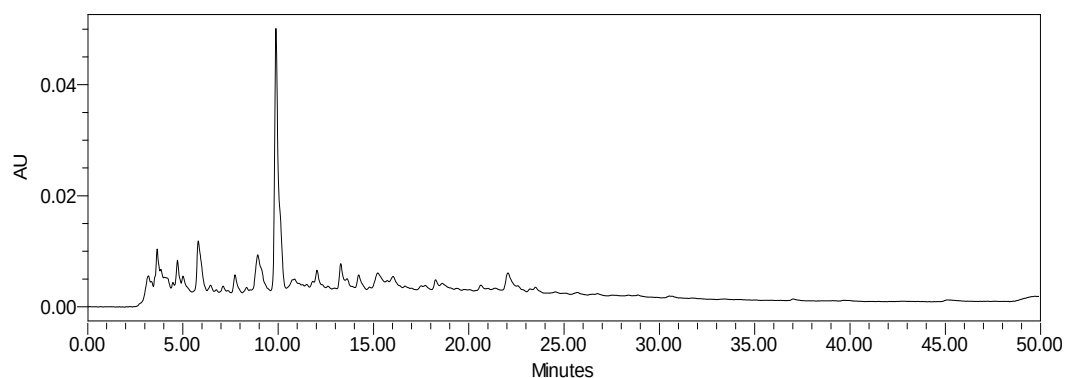


Figure B.125: Representative HPLC chromatograms of artichoke vinegar sample at 280 nm.

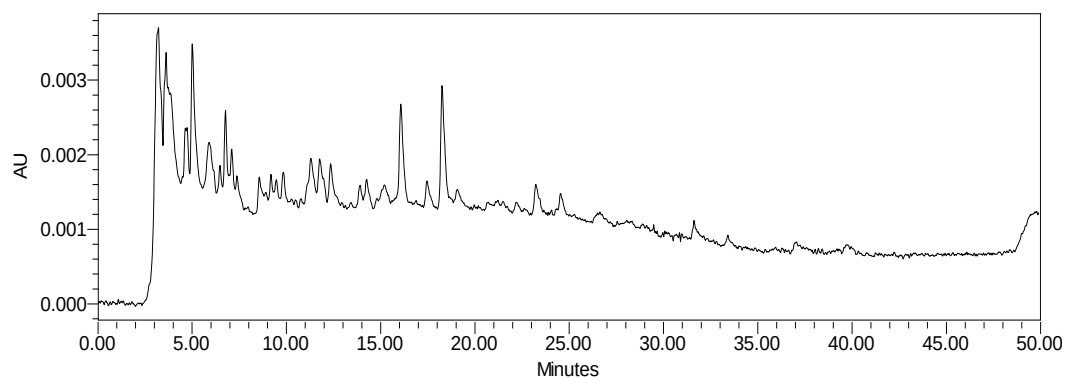


Figure B.126: Representative HPLC chromatograms of artichoke vinegar sample at 312 nm.

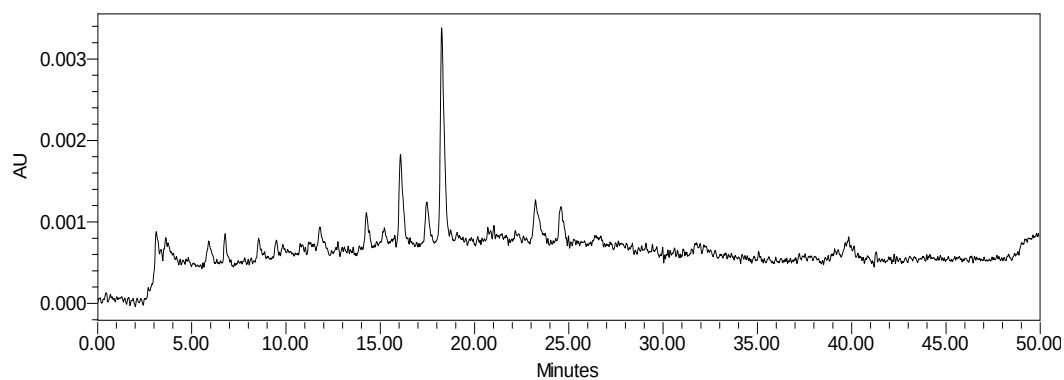


Figure B.127: Representative HPLC chromatograms of artichoke vinegar sample at 360 nm.

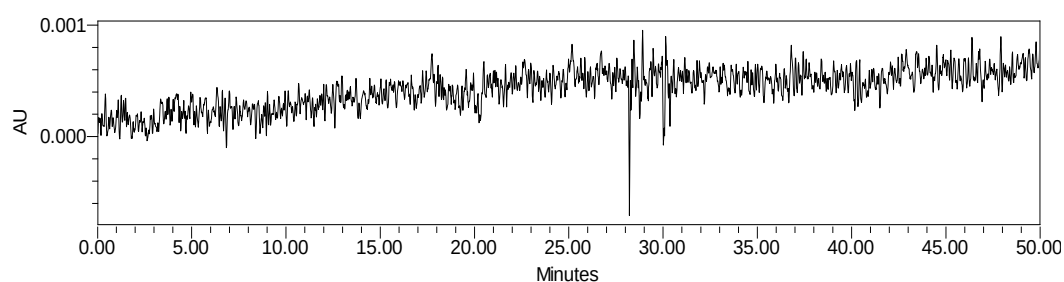


Figure B.128: Representative HPLC chromatograms of artichoke vinegar sample at 520 nm.

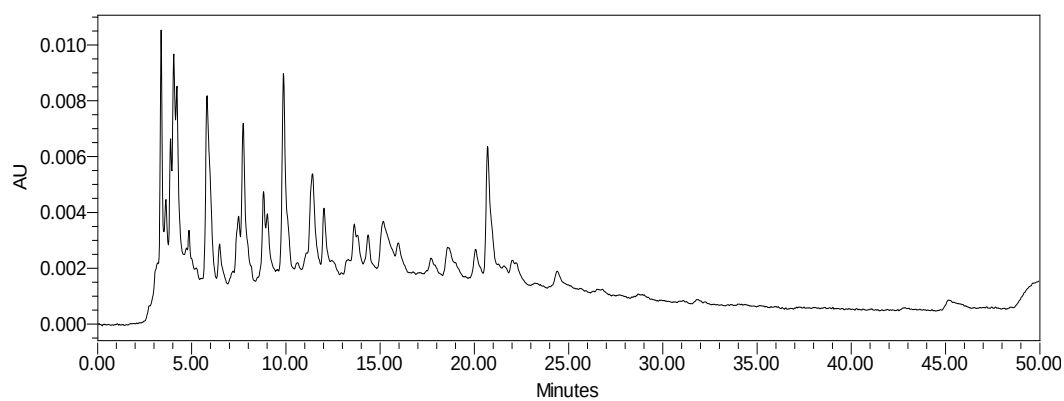


Figure B.129: Representative HPLC chromatograms of mulberry vinegar sample at 280 nm.

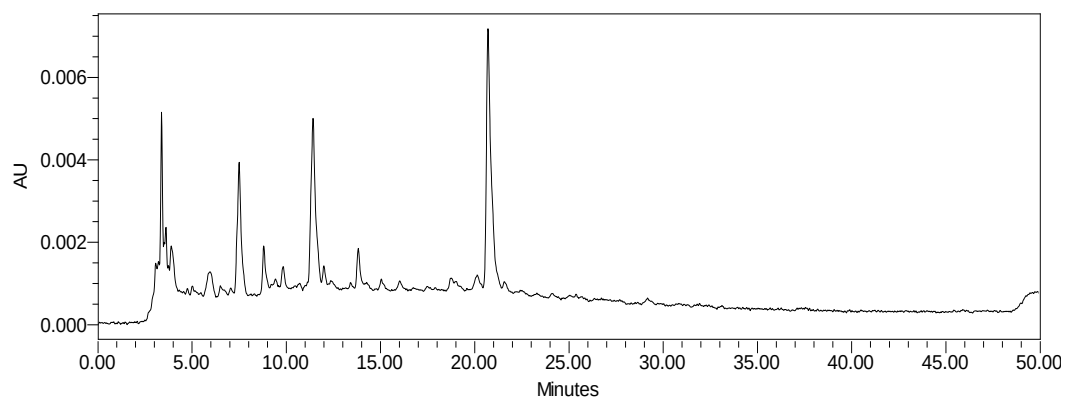


Figure B.130: Representative HPLC chromatograms of mulberry vinegar sample at 312 nm.

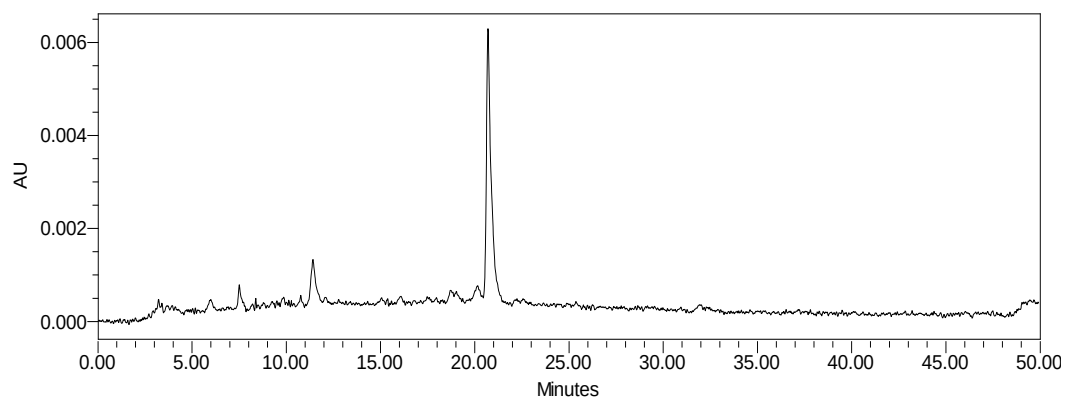


Figure B.131: Representative HPLC chromatograms of mulberry vinegar sample at 360 nm.

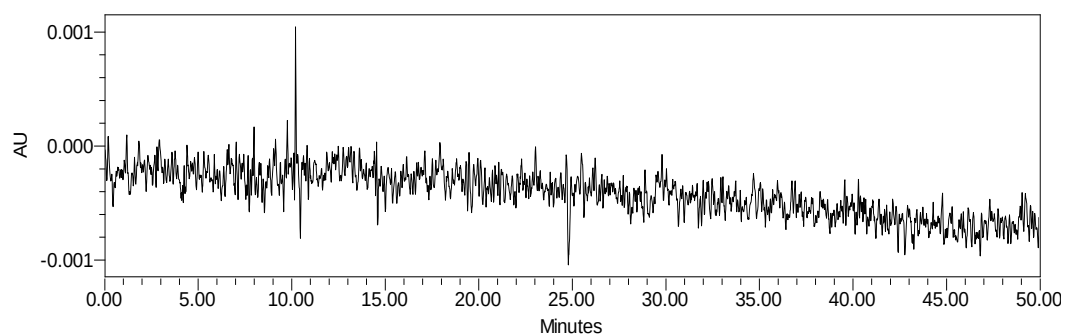


Figure B.132: Representative HPLC chromatograms of mulberry vinegar sample at 520 nm.

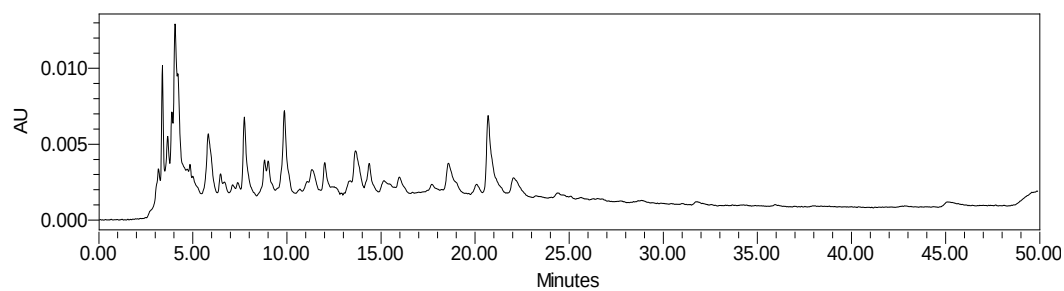


Figure B.133: Representative HPLC chromatograms of rice vinegar sample at 280 nm.

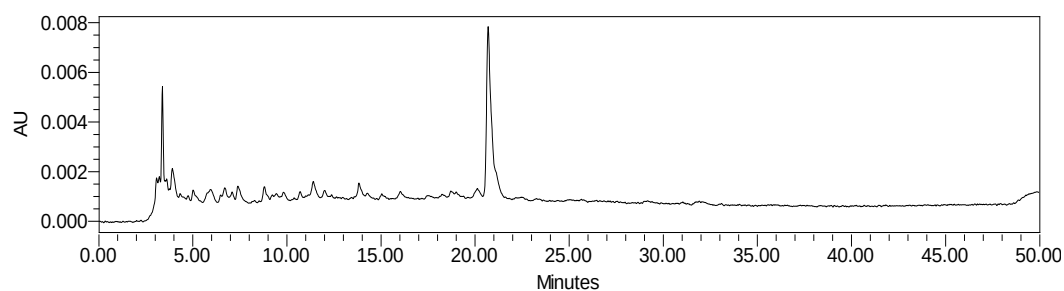


Figure B.134: Representative HPLC chromatograms of rice vinegar sample at 312 nm.

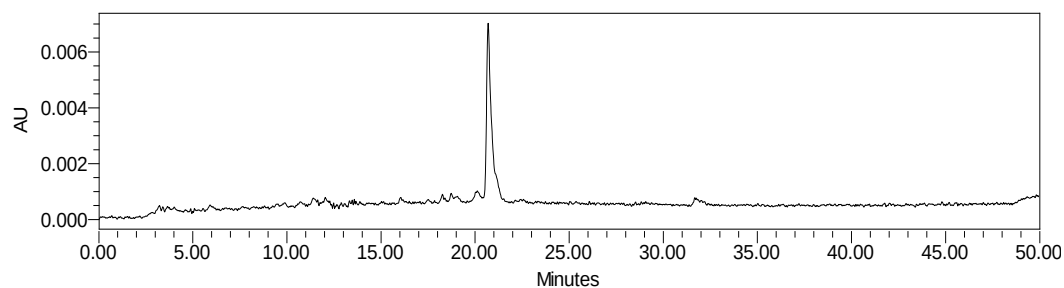


Figure B.135: Representative HPLC chromatograms of rice vinegar sample at 360 nm.

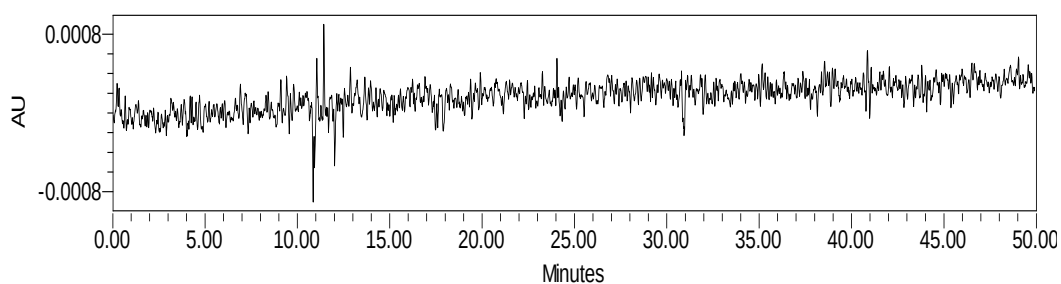


Figure B.136: Representative HPLC chromatograms of rice vinegar sample at 520 nm.

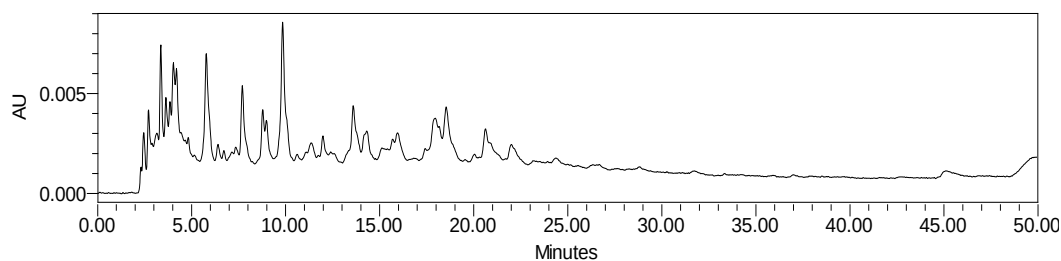


Figure B.137: Representative HPLC chromatograms of apricot vinegar sample at 280 nm.

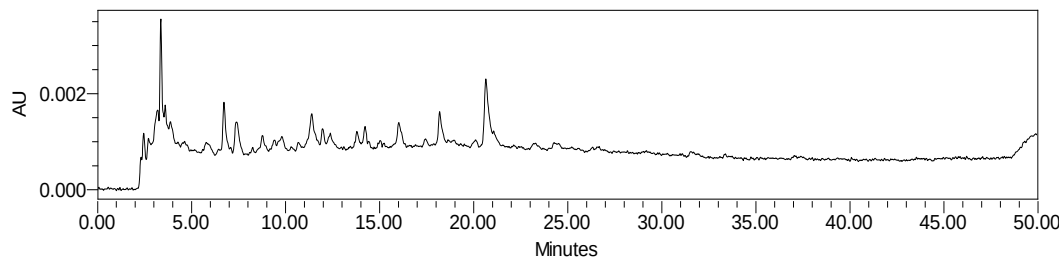


Figure B.138: Representative HPLC chromatograms of apricot vinegar sample at 312 nm.

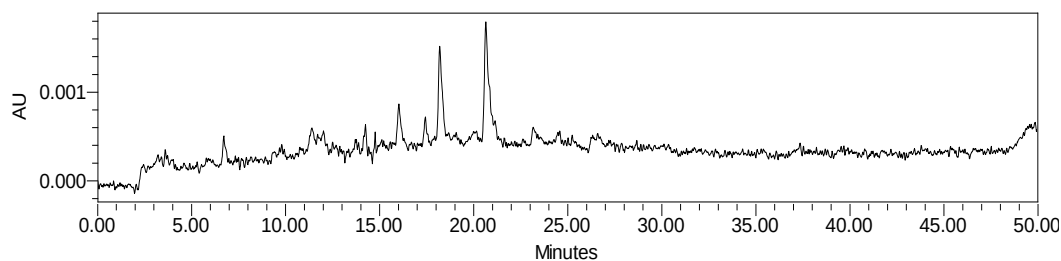


Figure B.139: Representative HPLC chromatograms of apricot vinegar sample at 360 nm.

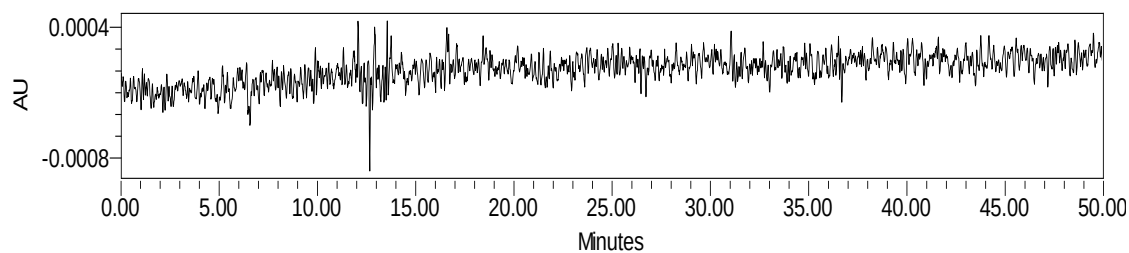


Figure B.140: Representative HPLC chromatograms of apricot vinegar sample at 520 nm.

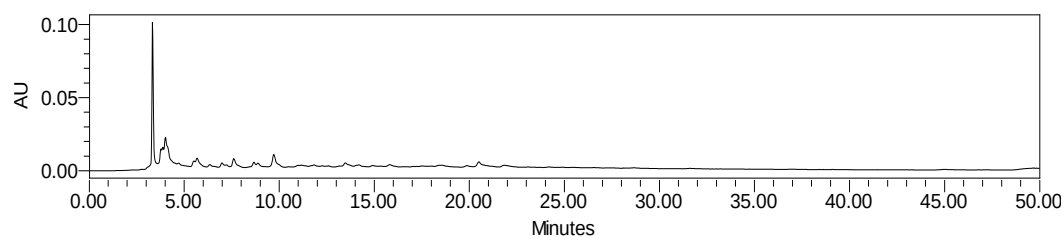


Figure B.141: Representative HPLC chromatograms of date vinegar sample at 280 nm.

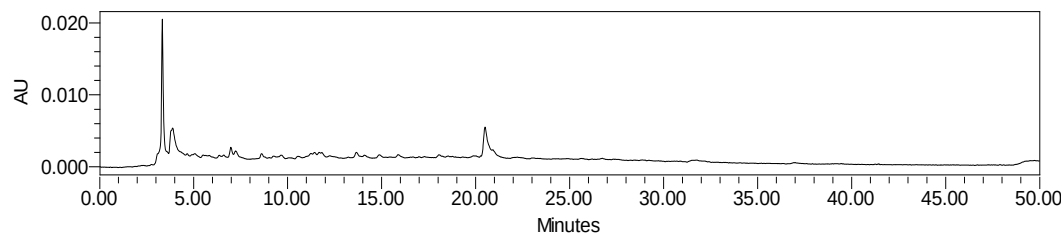


Figure B.142: Representative HPLC chromatograms of date vinegar sample at 312 nm.

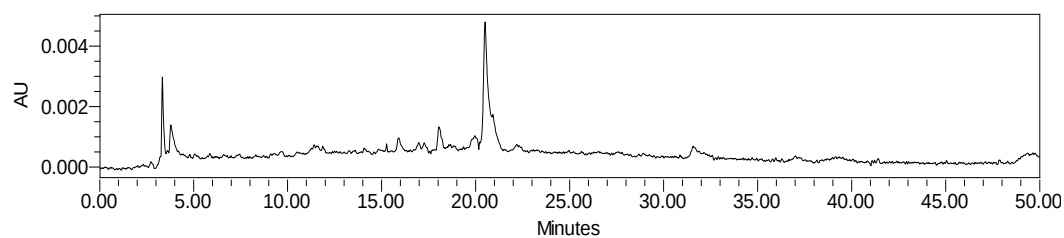


Figure B.143: Representative HPLC chromatograms of date vinegar sample at 360 nm.

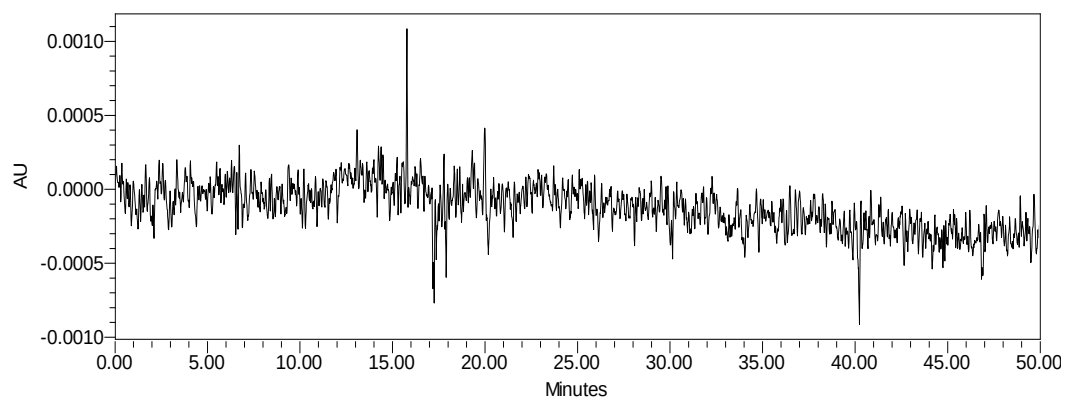


Figure B.144: Representative HPLC chromatograms of date vinegar sample at 520 nm.

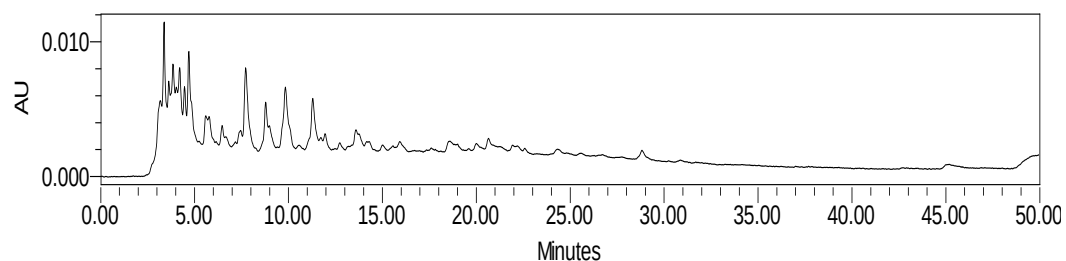


Figure B.145: Representative HPLC chromatograms of howthorn vinegar sample at 280 nm.

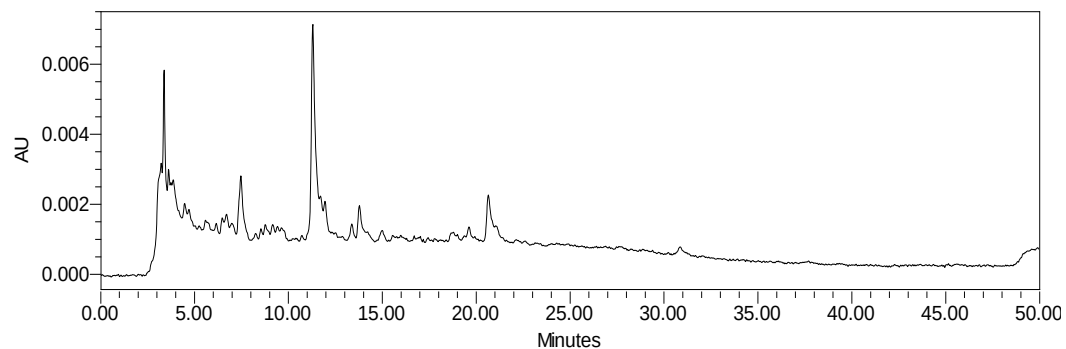


Figure B.146: Representative HPLC chromatograms of howthorn vinegar sample at 312 nm.

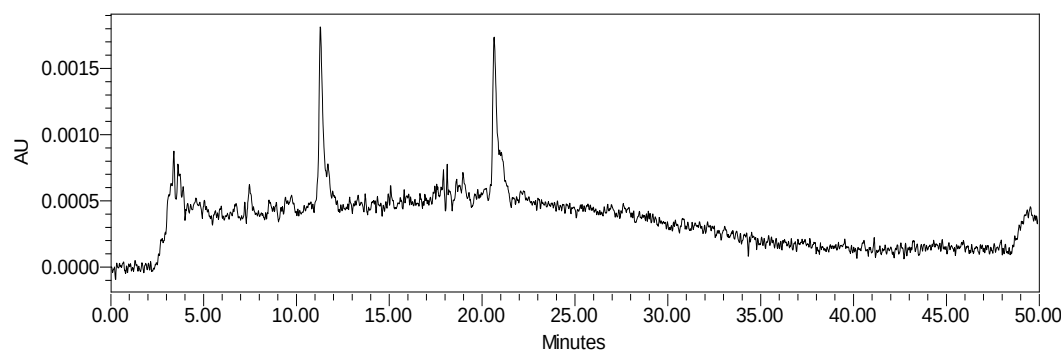


Figure B.147: Representative HPLC chromatograms of howthorn vinegar sample at 360 nm.

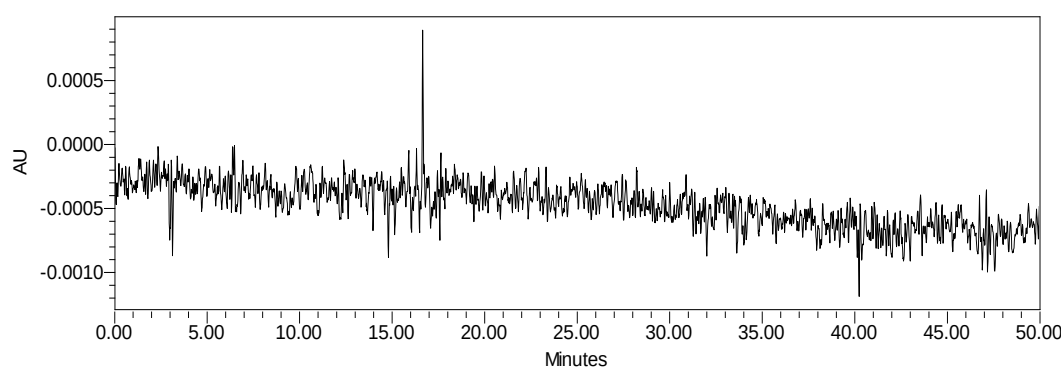


Figure B.148: Representative HPLC chromatograms of howthorn vinegar sample at 520 nm.

APPENDIX C

TABLE C. 1: Statistical analyses results of grape vinegar process step samples at fresh weight based.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	139,059	4	34,765	31,363	,000
	Within Groups	43,230	39	1,108		
	Total	182,289	43			
TFE	Between Groups	8379,836	4	2094,959	53,580	,000
	Within Groups	1564,000	40	39,100		
	Total	9943,836	44			
ABTS	Between Groups	70031,782	4	17507,945	6,258	,001
	Within Groups	111911,951	40	2797,799		
	Total	181943,733	44			
CUPRAC	Between Groups	10751,019	4	2687,755	14,796	,000
	Within Groups	7265,982	40	181,650		
	Total	18017,000	44			
DPPH	Between Groups	11162,173	4	2790,543	289,026	,000
	Within Groups	386,200	40	9,655		
	Total	11548,373	44			
FRAP	Between Groups	752,941	4	188,235	61,763	,000
	Within Groups	121,908	40	3,048		
	Total	874,849	44			

TABLE C. 2: Statistical analyses results of grape vinegar process step samples at dry weight based.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	44445,940	4	11111,485	27,910	,000
	Within Groups	15924,844	40	398,121		
	Total	60370,783	44			
TFE	Between Groups	1332848,484	4	333212,121	26,179	,000
	Within Groups	509119,422	40	12727,986		
	Total	1841967,906	44			
ABTS	Between Groups	10282469,672	4	2570617,418	6,073	,001
	Within Groups	16932632,387	40	423315,810		
	Total	27215102,060	44			
CUPRAC	Between Groups	5558838,644	4	1389709,661	33,313	,000
	Within Groups	1626966,123	39	41717,080		
	Total	7185804,768	43			
DPPH	Between Groups	4401505,067	4	1100376,267	75,427	,000
	Within Groups	583543,138	40	14588,578		
	Total	4985048,205	44			
FRAP	Between Groups	477119,323	4	119279,831	67,323	,000
	Within Groups	70869,902	40	1771,748		
	Total	547989,225	44			

TABLE C. 3: Statistical analyses results of apple vinegar process step samples at fresh weight based.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	4449,404	4	1112,351	475,208	,000
	Within Groups	81,927	35	2,341		
	Total	4531,331	39			
TFE	Between Groups	93502,348	4	23375,587	647,005	,000
	Within Groups	1336,770	37	36,129		
	Total	94839,118	41			
ABTS	Between Groups	15341579,148	4	3835394,787	657,439	,000
	Within Groups	204184,554	35	5833,844		
	Total	15545763,702	39			
CUPRAC	Between Groups	1417048,810	4	354262,203	354,672	,000
	Within Groups	36957,235	37	998,844		
	Total	1454006,045	41			
DPPH	Between Groups	4121526,529	4	1030381,632	2561,459	,000
	Within Groups	14481,489	36	402,264		
	Total	4136008,019	40			
FRAP	Between Groups	213698,586	4	53424,646	1560,239	,000
	Within Groups	1266,929	37	34,241		
	Total	214965,515	41			

TABLE C. 4: Statistical analyses results of apple vinegar process step samples at dry weight based.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	237172,229	4	59293,057	460,268	,000
	Within Groups	4508,804	35	128,823		
	Total	241681,033	39			
TFE	Between Groups	347000,679	4	86750,170	48,867	,000
	Within Groups	65683,868	37	1775,240		
	Total	412684,547	41			
ABTS	Between Groups	26906398,118	4	6726599,529	264,152	,000
	Within Groups	891272,128	35	25464,918		
	Total	27797670,246	39			
CUPRAC	Between Groups	6026184,520	4	1506546,130	102,509	,000
	Within Groups	499688,373	34	14696,717		
	Total	6525872,893	38			
DPPH	Between Groups	5351800,702	4	1337950,176	137,950	,000
	Within Groups	349157,631	36	9698,823		
	Total	5700958,333	40			
FRAP	Between Groups	483736,204	4	120934,051	272,214	,000
	Within Groups	16437,657	37	444,261		
	Total	500173,861	41			

TABLE C. 5: Statistical analyses results of grape vinegar *in vitro* bioaccessibility at fresh weight basis.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	58,451	3	19,484	69,531	,000
	Within Groups	8,967	32	,280		
	Total	67,418	35			
TFE	Between Groups	121,216	3	40,405	3,011	,046
	Within Groups	402,557	30	13,419		
	Total	523,773	33			
ABTS	Between Groups	14954,105	3	4984,702	422,130	,000
	Within Groups	377,871	32	11,808		
	Total	15331,975	35			
CUPRAC	Between Groups	7578,448	3	2526,149	45,455	,000
	Within Groups	1722,829	31	55,575		
	Total	9301,277	34			
DPPH	Between Groups	3065,707	3	1021,902	188,487	,000
	Within Groups	157,227	29	5,422		
	Total	3222,934	32			
FRAP	Between Groups	6537,009	3	2179,003	449,859	,000
	Within Groups	155,000	32	4,844		
	Total	6692,008	35			

TABLE C. 6: Statistical analyses results of apple vinegar *in vitro* bioaccessibility on fresh weight basis.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	10,653	3	3,551	10,688	,000
	Within Groups	10,631	32	,332		
	Total	21,284	35			
TFE	Between Groups	111,247	3	37,082	5,325	,005
	Within Groups	188,009	27	6,963		
	Total	299,256	30			
ABTS	Between Groups	5696,319	3	1898,773	121,570	,000
	Within Groups	499,800	32	15,619		
	Total	6196,119	35			
CUPRAC	Between Groups	5708,793	3	1902,931	63,248	,000
	Within Groups	962,772	32	30,087		
	Total	6671,566	35			
DPPH	Between Groups	549,142	3	183,047	36,985	,000
	Within Groups	138,580	28	4,949		
	Total	687,722	31			
FRAP	Between Groups	3771,823	3	1257,274	214,047	,000
	Within Groups	182,089	31	5,874		
	Total	3953,912	34			

TABLE C. 7: Statistical analyses results of pomegranate vinegar *in vitro* bioaccessibility on fresh weight basis.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	715,954	3	238,651	57,690	,000
	Within Groups	124,104	30	4,137		
	Total	840,058	33			
TFE	Between Groups	26089,535	3	8696,512	494,051	,000
	Within Groups	563,279	32	17,602		
	Total	26652,814	35			
ABTS	Between Groups	201920,225	3	67306,742	522,807	,000
	Within Groups	4119,712	32	128,741		
	Total	206039,938	35			
CUPRAC	Between Groups	357741,673	3	119247,224	49,788	,000
	Within Groups	76643,453	32	2395,108		
	Total	434385,126	35			
DPPH	Between Groups	115119,577	3	38373,192	252,177	,000
	Within Groups	3956,359	26	152,168		
	Total	119075,936	29			
FRAP	Between Groups	170514,070	3	56838,023	537,781	,000
	Within Groups	3382,076	32	105,690		
	Total	173896,145	35			

TABLE C. 8: Statistical analyses results of balsamic vinegar *in vitro* bioaccessibility on fresh weight basis.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	40355,435	3	13451,812	137,630	,000
	Within Groups	3127,637	32	97,739		
	Total	43483,072	35			
TFE	Between Groups	258956,607	3	86318,869	320,472	,000
	Within Groups	8349,825	31	269,349		
	Total	267306,431	34			
ABTS	Between Groups	525652,894	3	175217,631	334,148	,000
	Within Groups	16779,878	32	524,371		
	Total	542432,772	35			
CUPRAC	Between Groups	2001705,001	3	667235,000	159,870	,000
	Within Groups	129382,187	31	4173,619		
	Total	2131087,188	34			
DPPH	Between Groups	421197,078	3	140399,026	226,955	,000
	Within Groups	17939,979	29	618,620		
	Total	439137,057	32			
FRAP	Between Groups	840403,024	3	280134,341	745,570	,000
	Within Groups	9393,300	25	375,732		
	Total	849796,324	28			

TABLE C. 9: Statistical analyses results of different vinegar samples on fresh weight basis.

		Sum of Squares	df	Mean Square	F	Sig.
TFL	Between Groups	74529,726	17	4384,102	132,644	,000
	Within Groups	4759,451	144	33,052		
	Total	79289,178	161			
TFE	Between Groups	458355,989	17	26962,117	201,058	,000
	Within Groups	19310,617	144	134,102		
	Total	477666,606	161			
ABTS	Between Groups	1339900,142	17	78817,655	62,690	,000
	Within Groups	181046,152	144	1257,265		
	Total	1520946,294	161			
CUPRAC	Between Groups	3838819,433	17	225812,908	97,617	,000
	Within Groups	330795,619	143	2313,256		
	Total	4169615,051	160			
DPPH	Between Groups	4364677,686	17	256745,746	461,059	,000
	Within Groups	80187,931	144	556,861		
	Total	4444865,617	161			
FRAP	Between Groups	1509808,344	17	88812,256	179,045	,000
	Within Groups	71428,927	144	496,034		
	Total	1581237,270	161			

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Publications/Presentations on Thesis

Bakır, S., Çapanoğlu-Güven, E. (2013). Türkiye’de Yaygın Olarak Tüketilen Bazı Sirke Çeşitlerinin Antioksidan Özelliklerinin İncelenmesi, TGDF Gıda Kongresi, Antalya.