DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

STUDY OF AROMA PROFILES AND PHENOLIC CONTENT OF TURKISH GOJI BERRIES BY SPME-GC

by

Samad Babatope IBRAHIM

July, 2021

İZMİR

STUDY OF AROMA PROFILES AND PHENOLIC CONTENT OF TURKISH GOJI BERRIES BY SPME-GC

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **STUDY OF AROMA PROFILES AND PHENOLIC CONTENT OF TURKISH GOJI BERRIES BY SPME-GC** completed by **SAMAD BABATOPE IBRAHIM** under supervision of **PROF. DR. MELEK MERDIVAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Samad Babatope IBRAHIM

STUDY OF AROMA PROFILES AND PHENOLIC CONTENT OF TURKISH GOJI BERRIES BY SPME-GC

ABSTRACT

Goji berry (GB) is a powerful antioxidant used in Chinese medicine to reduce cholesterol, lower blood pressure, and clean blood. GB is an authentic fruit with orange, red color and tangy flavor. It is aimed to determine the volatile profile, phenolic compounds, elemental compositions and antioxidant activity of the dried GB samples of Turkish origin which has been increasingly used all over the world in recent years.

The volatile organic compounds found in GB samples were extracted using headspace-solid phase microextraction and determined by gas chromatography-mass spectrometer using commercial SPME fiber. The phenolic compounds found in GB samples were analyzed by HPLC. The elemental composition of the GB sample was determined with ICP-OES following acid digestion. Antioxidant activity of GB sample was determined by DPPH based method. The volatile organics of GB sample were also determined by hydrodistillation method.

The extraction efficiencies of polyacrylate and divinylbenzene/carboxen/ polydimethylsiloxane SPME fibers were found higher than the hydrodistillation method. In SPME method, the extraction temperature and extraction time were optimized as 60 ^{o}C and 60 min, respectively. Some identified compounds are safranal, nonanal, geranyl acetone, benzophenone, fatty acids like ethyl palmitate, methyl oleate, methyl linoleate, etc. Na, K, Mg, Ca and P as macro elements, Fe, Cu, Mn, Zn, Se, V as minor elements and Cd, Hg, and Pb as trace elements were determined in the GB samples. These elements and the phenolic compounds showed similar contents with the previous works. The black GB sample has the highest antioxidant activity.

Keywords: Goji berry, HS-SPME-GC/MS, volatile compounds, phenolic compounds, element compositions, antioxidant activity

SPME-GC İLE TÜRK GOJİ MEYVELERİNİN AROMA PROFİLLERİNİN İNCELENMESİ

ÖZ

Goji beri (GB), Çin tıbbında kolesterolü düşürmek, kan basıncını düşürmek ve kanı temizlemek için kullanılan güçlü bir antioksidandır. GB, turuncu, kırmızı rengi ve keskin aroması olan otantik bir meyvedir. Son yıllarda tüm dünyada kullanımı giderek artan Türk menşeyli kurutulmuş GB örneklerinin uçucu profili, fenolik bileşikleri, elementel bileşimleri ve antioksidan aktivitelerinin belirlenmesi amaçlanmıştır.

GB örneklerinde bulunan uçucu organik bileşikleri, ticari KFME fiber kullanılarak tepe boşluklu - katı faz mikro ekstraksiyon yöntemi ile ekstre edildi ve gaz kromatografisi-kütle spektrometresi ile belirlendi. GB örneklerinin fenolik bileşikleri HPLC kullanılarak belirlendi. GB örneklerinin element bileşimi, asitle çözündürdükten sonra ICP-OES ile analiz edildi. GB örneklerinin antioksidan kapasitesi DPPH'ye dayalı yöntem ile belirlendi. GB örneklerinin uçucu bileşimi ayrıca hidrodistilasyon yöntemi ilede belirlendi.

Poliakrilat ve divinilbenzen/karboksen/polidimetilsiloksan KFME fiberlerinin ekstraksiyon verimleri hidrodistilasyon yönteminden daha yüksek bulunmuştur. KFME yöntemi için optimum ekstraksiyon sıcaklığı ve ekstraksiyon süresi sırasıyla 60 ^{o}C ve 60 dak olarak belirlendi. Tanımlanan bazı bileşikler safranal, nonanal, geranil aseton, benzofenon, etil palmitat, metil oleat, metil linoleat vb. gibi yağ asitleridir. GB örneklerinde, makro elementler olarak Na, K, Mg, Ca ve P, minor element olarak Fe, Cu, Mn, Zn, Se ve V ve eser element olarak Pb, Hg ve Cd belirlendir. Bu elementler ve fenolik bileşikler önceki çalışmalarla benzer içerikler göstermiştir. Siyah GB örneği en yüksek antioksidan aktiviteye sahiptir.

Anahtar kelimeler: Goji beri, TB-KFME-GC/MS, uçucu bileşikler, fenolik bileşikler, element bileşimi, antioksidant aktivitesi

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CHAPTER ONE

INTRODUCTION

1.1 Goji Berry

Goji berry fruits are herbal medicinal plants from *Lycium* plants popularly known for the treatment of diseases since time immemorial in China. Its Botanical name, *Lycium barbarum* was allocated by Carolus Linnaeus, the Swedish botanist. The name of the species as *barbarum* was given by him (Dharmananda, 2007). *L. barbarum* fits in the plant family which include tomatoes, potatoes, eggplants, and peppers. It grows in Asia countries such as China, Tibet. Its fruits length is around 1–2 cm, and it looks like bright orange-red ellipsoid berries (Figure 1.1) (Bryan, Costa, Giese, Nummy, Rapp, & Seamon, 2008). The inception habitat of goji is not known. However, it was said that nearly "90% of the commercially available goji berries around the world are produced by *Lycium barbarum"*, which came from China (Zhang et al., 2016). In addition, it was stated that "approximately 70 species of *Lycium* grew in different regions such as North America, South America, Southern Africa, Eurasia, and Australia" (Fukuda, Yokohama, & Ohashi, 2001). It has different names such as Chinese wolfberry, Barbary wolfberry, boxthorn and many more (Bryan, Costa, Giese, Nummy, Rapp, & Seamon, 2008; PDR, 2007). The fruit has long been known to be used as a functional food for many years. Also, they have organoleptic properties, such as color, flavor, odor, and aroma (Bensky, Gamble, & Kaptchuk 1993; Bryan, Costa, Giese, Nummy, Rapp, & Seamon, 2008; Chang & But 2001; Zhu, 1998). From several literatures done on *L. barbarum*, it showed that the traditional properties had lots of positive effects compared to its other species, these include anti-aging, neuroprotection, endurance, high digestion, blood-sugar control, fight against oxidation, etc. (Bensky et al., 1993; Bryan, Costa, Giese, Nummy, Rapp, & Seamon, 2008). Goji berry contains essential oils, phenolic compounds, antioxidants, and essential elements which makes it to fulfil this beneficial role.

Figure 1.1 Pictures of Lycium barbarum fruit (Amagase & Farnsworth, 2011)

Essential oils, also called volatile oils, are evanescent chemical compounds with sweet scent and vapourization process, that is produced by hydro-distillation. Their physical properties include most are colorless or light yellow, aromatic odor or caustic smell, indissoluble in water but dissolve totally in organic compounds such as anhydrous ethanol, ether, (Zhang et al., 2021) etc. They are plant minor products found in the intrinsic hairs, secretory cells and are spread into roots, stems, leaves, and other parts of the plants. According to Zhang et al., (2021), essential oils can be divided into different organic groups which include terpenoids, alcohols, esters, carbonyl compounds, etc. Since the ancient times, they were known for treating emotional diseases in the world as an anti-depressant (Zhang et al., 2021). Examples of essential oils include safranal, linalool, methyl salicylate, (*E*)-geranylacetone, myristic acid, 2-pentadecanone, linoleic acid, etc. (Altinas, Koşar, Kirimer, Başer, & Demirci, 2006).

Phenols and flavonoids are biologically active organic compounds present in goji berries and have many health utilities (Gan, Zhang, Yang, & Xu, 2004; Sa et al., 2019). Phenolic compounds which are derived from benzoic and cinnamic acids are secondary plant metabolites. Recently, they played a significant role in the fight

against cancer and heart diseases. It was explained that the antioxidant activity of goji berries was higher than that of vitamin antioxidants which may be attributed to the success. The most prevalent form of phenolic compounds is p-hydroxybenzoic acid, ferulic acid, procatechuic acid, caffeic acid, vanillic acid and p-coumaric acid which are often found in vegetables and fruits as glycosides or esters (Han et al., 2001). Phenolic compounds were separated from flavonoids with some of the extraction techniques by high performance liquid chromatography-diode array detector (HPLC-DAD) and liquid chromatography-mass spectrometer (LC-MS) (Molnar-Perl & Fuzfai, 2005; Sa et al., 2017). Some examples of their chemical structures are shown in Figure 1.2 below.

Goji berries and their derived products are undoubtedly an important source of natural antioxidants which contributed hugely to their nutritional quality (Yao, Heinrich, & Weckerle, 2018). Antioxidants are compounds that inhibit oxidation from damaging the cell organisms. Polyphenols, carotenoids, and polysaccharides are different types of antioxidants (Zhang, Chen, Zhao, & Xi, 2016). Polyphenols contain flavonoids and phenolics, which act as radical scavengers and antioxidants for the fight against cancer, cardiovascular disease, neurodegeneration, and other chronic diseases (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012). Dried goji berry fruits are composed of carotenoids, of which zeaxanthin dipalmitate represents the main molecule – 30% to 80% of the total carotenoids (Patsilinakos, Ragno, Carradori, Petralito, & Cesa, 2018). Carotenoids are known for protecting the eyes from visual impairment, lutein and zeaxanthin which are the most represented pigments in the macula region of human retina protect the macula from oxidative damage by scavenging harmful reactive oxygen species (Kim, Kim, Kwon, & Chang, 2018). *Lycium barbarum* polysaccharides, LBP are the most researched components from goji fruits – contain 5 to 8% of the dried fruits. They control blood sugar glucose, modulate glucose metabolism which improves oxidative stress markers and, ease insulin resistance (Masci et al., 2018).

Goji fruits have many kinds of minerals as macro elements (Calcium, Potassium, Magnesium, Sodium and Phosphorus) and micro elements (Aluminum, Arsenic, Barium, Cadmium, Cobalt, Chromium, Copper, Iron, Manganese, Molybdenum, Nickel, Lead, Antimony, Selenium, Tin, Strontium, Vanadium and Zinc). These

elements have significant biological properties and are also found at high level in other species belonging to the Solanaceae family (Fattore et al., 2016). The macro elements have several physiological functions, membrane depolarization, water control, nucleic acids in protein synthesis, human reproduction and many more (Akh, 1998; Gröber, Schmidt, & Kisters, 2015). The micro elements are useful for many biochemical reactions such as enzymatic cofactors, growth, gene expression, fertility. They are important for patients having iron deficiency, stimulates the immune system and protect the heart from heart attacks and strokes etc. (Osredkar & Sustar, 2011; Gruzewska, Michno, Pawelczyk, & Bielarczyk, 2014; Maret, 2016; Rayman, 2000). Goji berry is an important herbal plant as a result of all these rich constituents it has been recorded that it helped in nourishing the liver, kidney, brightens the eyes, protect the cells against oxidative damages, supplement energy, blood and for many more medicinal benefits (Meng et al., 2019).

1.2 Solid-phase Microextraction (SPME)

Extraction is the process of separating a substance usually the analyte from the matrix. This technique is very essential to obtain volatile compounds from plants such as Goji berries etc. The conventional and new extraction methods include steam distillation and hydro-distillation, are basically the oldest known, soxhlet extraction (de Castro & Garcia-Ayuso, 1998; Ngamwonglumlert, Devahastin, & Chiewchan, 2017), super critical fluid extraction, Microwave assisted extraction (Azmir et al., 2013), pressured liquid extraction (Howard & pandjaitan, 2008), solid phase extraction (Cullere, Aznar, Cacho, & Ferreira, 2003; Lopez, Aznar, Cacho, & Ferreira, 2002), solid phase microextraction (SPME) – it was first developed by Arthur and Pawliszyn in 1990 compared with classicalclassical extraction methods, it was konwn as environmentally friendly due to the much fewer amounts of organic solvents needed (Cui, Gu, Jiang, Li, Wang, & Yan, 2009). Lately, extraction of volatiles in spices and herbs has been performed with the SPME method because it needs less amount of sample, economic, no solvent consumption, simple, fast and sufficient sensitivity (Dawidowicz et al., 2016).

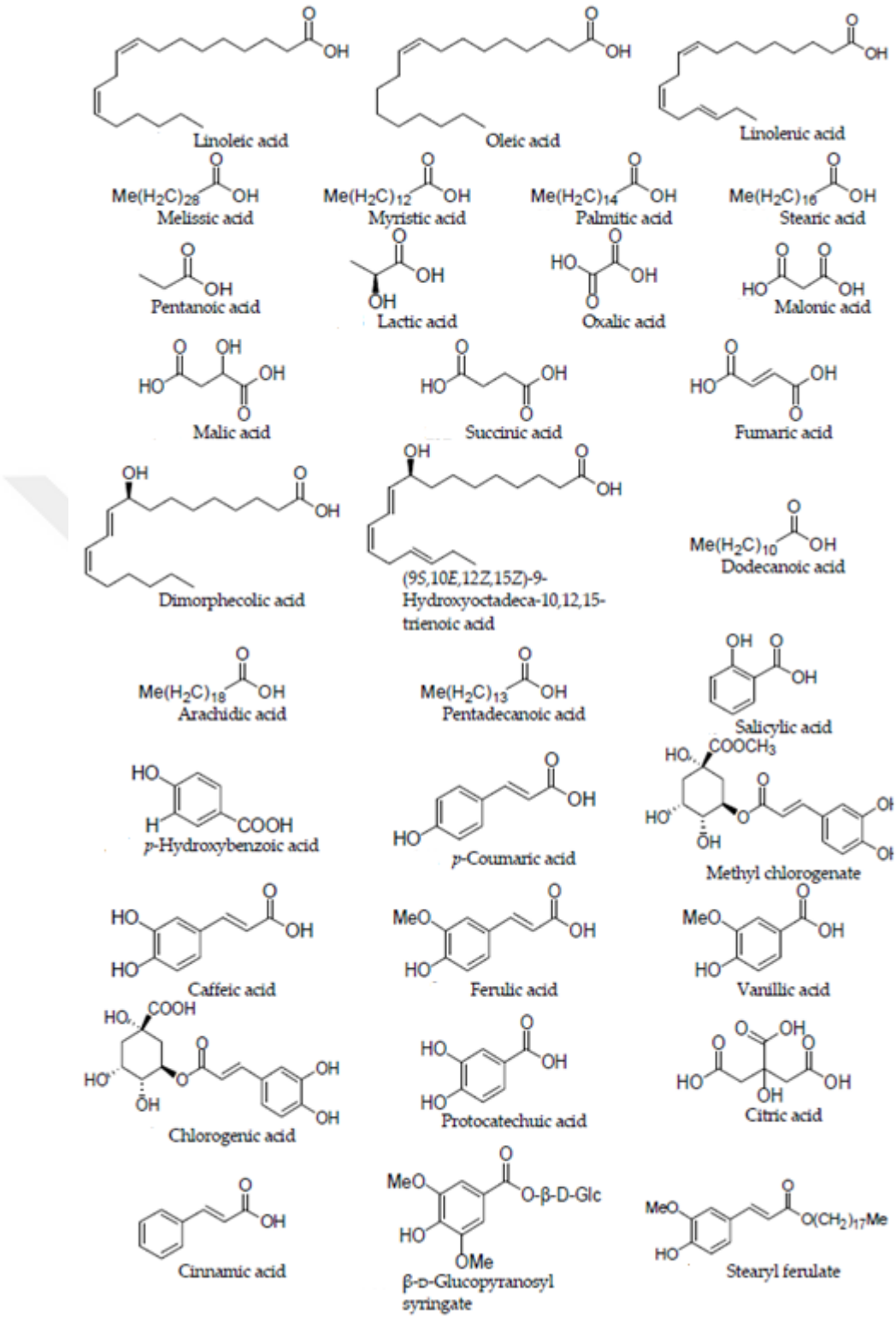

Figure 1.2 Chemical structures of essential oils and phenolic compounds (Qian, Zhao, Yang, & Huang, 2017)

1.2.1 Principle of SPME

Firstly, small amount of the sample matrix is incubated in an incubator for a specified time and temperature. During this period, an equilibrium is reached between the sample matrix and the extraction phase. At this moment, exposing the fiber for extra time does not attract more analytes. SPME technique is of 2 kinds – association with tube and fiber designs. The one with coated fibers is of main interest. The first commercially available SPME fiber device is shown in Figure 1.3. Extraction starts once the coated fiber is inserted into the sample and is complete when equilibrium is reached between the sample matrix and the coated fiber (Pawliszyn, 1997).

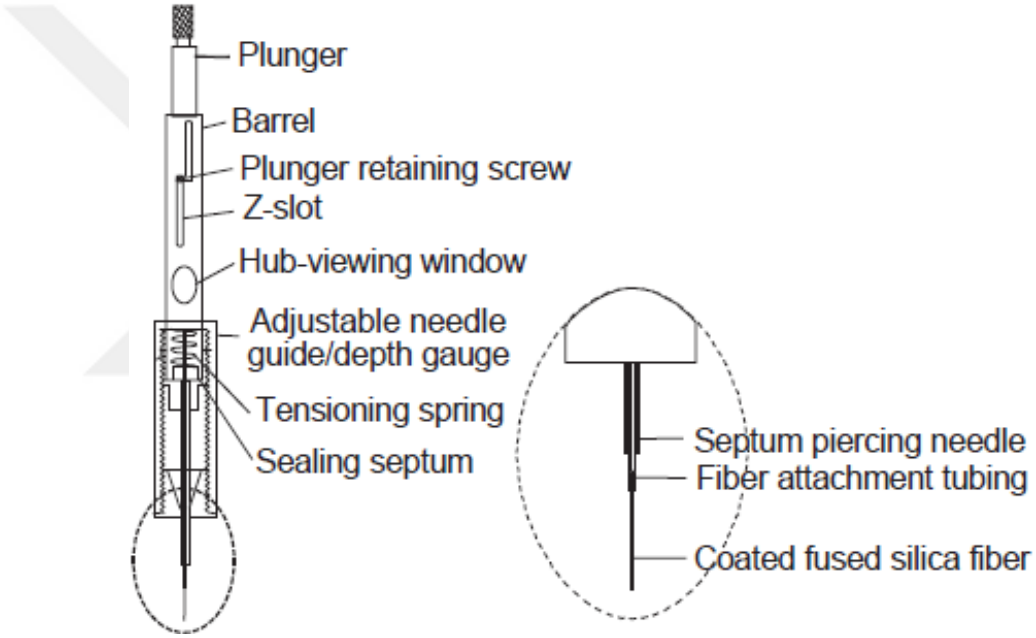

Figure 1.3 SPME device produced by Supelco (Pawliszyn, 1997)

1.2.2 Commercial Coating Fibers

The coating fibers for SPME are divided into four parts: the type of coating, the coating thickness, the polarity, and if the coating is absorbent or adsorbent as shown in Table 1.1 and their chemical structures are shown in Figure 1.4 as well. The polarity of the coating is determined by the type of phase involved. All the SPME fibers are bipolar to some extent (polar and non-polar) because they extract non-polar polar and polar compounds, but polarity is decided depending on the entire coating material property. The thicker the coating fibers, the more the volatile analytes are retained

while the high-molecular-weight analytes extraction are better with thin coating fibers (Shirey, 2012).

Table 1.1 Types of commercial SPME coating fibers (Shirey, 2012)

Type of coating	**Extraction mechanism**	**Polarity**
7 µm PDMS	Absorbent	Non-polar
30 µm PDMS	Absorbent	Non-polar
100 µm PDMS	Absorbent	Non-polar
85 µm PA	Absorbent	Polar
60 µm PEG (Carbowax)	Absorbent	Polar
65 µm PDMS-DVB	Adsorbent	Bipolar
15 µm Carbopack Z-PDMS	Adsorbent	Bipolar
55µm/30 µm DVB/Carboxen-PDMS	Adsorbent	Bipolar
85 µm Carboxen-PDMS	Adsorbent	Bipolar

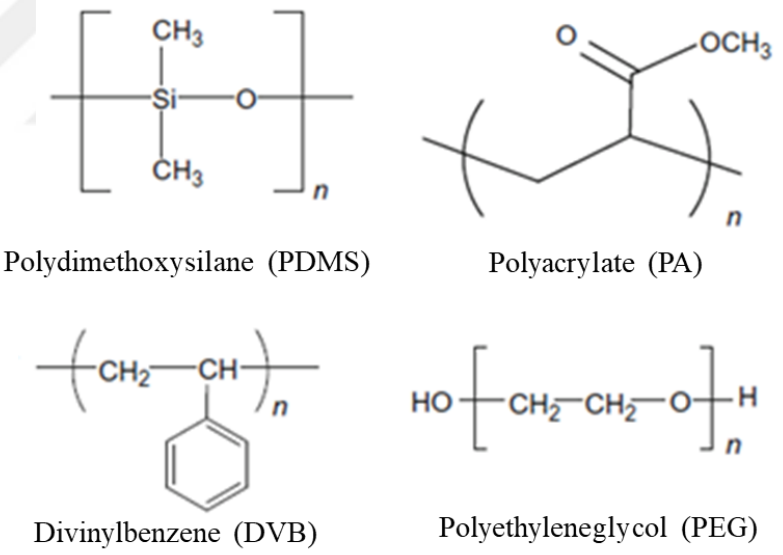

Figure 1.4 Chemical structures of common polymers used as SPME coatings (Shirey, 2012)

1.2.3 Types of SPME Extraction Method

There are three basic types of extractions that can be performed using SPME: Direct, Headspace, and Membrane extractions. Direct and Headspace extractions are the common types but in this study Headspace SPME is of main interest.

1.2.3.1 Direct Extraction

In this technique, the coated fiber is inserted into the sample matrix and the analytes are moved directly into the extracting phase. According to Pawliszyn, (1997), to ensure quick extraction, some criteria are needed to be fulfilled. It was said that "for gaseous samples, free convection of air is enough to facilitate rapid balancing, and for aqueous matrices, more efficient agitation techniques, such as fast sample flow, rapid fiber, or vial movement, stirring, or sonication is required". These conditions are required to achieve proper extraction process (Pawliszyn,1997).

1.2.3.2 Headspace SPME (HS-SPME)

In this mode, the analytes are transported through the air barrier before they can reach the coating fiber. This alteration serves mainly to protect the fiber coating from the damage by high molecular mass and other nonvolatile interferences present in the sample matrix, such as humic materials or proteins. The headspace mode also allows modification of the matrix, such as a change of the pH, without damaging the fiber (Pawliszyn, 1997). Many books and review articles have been published about the use of the headspace SPME method for the analysis of volatile organics and food aroma (Jelen, 2006; Jelen, Majcher, & Dziadas, 2012; Kudlejova & Risticevic, 2009; Steffen & Pawliszyn, 1996; Zhu et al., 2013).

1.2.4 Principle of HS-SPME

This involves both extraction and GC-MS steps. First, the sampling vial is put into the heater block above the heater-magnetic stirrer. At the selected temperature, the vial is heated for a certain time and then the fiber is inserted into the vial for extraction. Besides, equilibrium in the process equilibrium is achieved between the sample, the headspace, and the fiber. By decreasing the headspace volume, the amount of material absorbed/adsorbed onto the fiber can be increased. Other measures of increasing sensitivity include the addition of salt and agitation of the sample (Steffen & Pawliszyn, 1996; Zhang & Pawliszyn, 1993). After extraction, the fiber is withdrawn and then desorbed in the injection port of the gas chromatography-mass spectrometry (GC-MS) to carry out analysis in Figure 1.5.

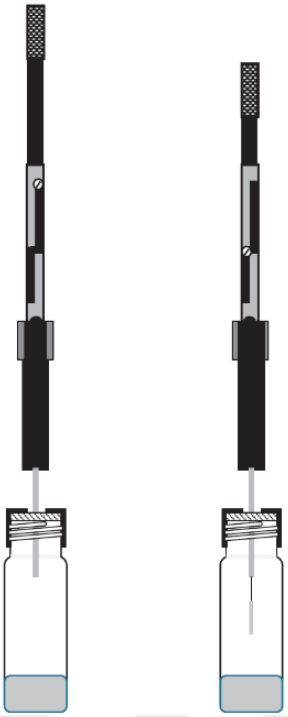

Figure 1.5 Fiber position as up and down positions in headspace-solid phase microextraction (Parker, Elmore, & Balagiannis 2015)

1.3 Related Studies

Lu and coworkers characterized the aroma volatile organic compounds found in Ningxia goji berry and also represented the interaction between fruit development and amount of aroma volatile compounds (Lu et al., 2017). The analysis of volatile organic compounds were performed by SPME method using divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber coupled to gas chromatography-mass spectrometer (GC/MS). In that study, a total of 193 volatiles were detected and 56 compounds were identified in the berries studied. These compounds were classified as 17 aldehydes, 5 terpenoids, 8 esters, 5 alcohols, 6 ketones, 2 furans, 3 carboxylic acids and 11 terpenes.

Meng and coauthors developed a new analytical method combined with statistic tools to determine the geographical origin of wolfberries from different provinces in China where head space-solid phase microextraction gas chromatography-combustion-isotope ratio mass spectrometry (HS-SPME GC-IRMS) was compared with the direct method GC-IRMS. Five types of SPME fiber were optimized for the study, out of which DVB/CAR/PDMS was selected because it had the highest yield.

HS-SPME GC-IRMS was chosen for the analysis since it was stable and reliable. The wolfberry volatile compounds (limonene, tetramethylpyrazine, safranal, geranylacetone, and β-ionone) found in these provinces were determined by HS-SPME GC-IRMS. Thus, making HS-SPME GC-IRMS method a suitable tool for the determination of the geographical origin of the berries (Meng et al., 2019).

Selected antioxidants and total antioxidant capacity of goji (*Lycium spp.*) berries were evaluated using α,α-diphenyl-β-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3 ethylbenzo-thiazolin-6-sulfonicacid) radical cation ($ABTS^{+}$), oxygen radical absorbance capacity (ORAC) and OH-radical scavenger capacity (OH-RSC) by Protti and co-authors (Protti et al., 2017). The antioxidant capacity (AC) values obtained with DPPH, ABTS, ORAC and OH-RSC assays varied from 13.9 to18.5 μmol vitamin C equivalents (VCE) g^{-1}, 54 to 61 μmol VCE g^{-1}, 180-260 μmol VCE g^{-1} and 70-630 μmol VCE g^{-1}, respectively. According to AC values, it was concluded that Goji berry has high nutritional and commercial value (Protti et al., 2017).

Sa and coauthors determined the phenolic compounds and flavonoids in dried fruits and capsules present in goji berries from Brazil. It was reported that out of the 13 phenolic compounds explored, 8 bioactive phenolic acids and 2 flavonoids were determined while the remaining compounds were below limit of quantification (LOQ). Furthermore, it was revealed that dried fruits had the highest contents. (Sa et al., 2019).

Also, they carried out a multi-elemental analysis experiment and the resulting solutions, quantitative analysis of 23 elements (Aluminum, Arsenic, Barium, Calcium, Cadmium, Cobalt, Chromium, Copper, Iron, Potassium, Magnesium, Manganese, Molybdenum, Sodium, Nickel, Phosphorus, Lead, Antimony, Selenium, Tin, Strontium, Vanadium and Zinc) were analyzed by Inductively coupled plasma-optical emission spectrometry (ICP-OES). Sa and coauthors stated that the values obtained for Calcium, Potassium, Magnesium, Sodium, Phosphorus and that of Copper, Iron, Manganese, Selenium, Vanadium and Zinc were within the limited range. However, the values for Copper and Manganese were lower than those found in other literatures by Llorent-Martínez, Fernandez-Decordova, Ortega-Barrales, & Ruiz-Medina, (2013); Skowron, Grzeskowiak, Stanisz, & Waskiewicz, (2017). According to the work done by Vigo, Narito, & Marques (2004), it was stated that "this could be due to

mineral changes in the soil and lower bioaccumulation of these minerals in the fruit". In addition, the values ($mgkg^{-1}$) for Vanadium, Zinc, Selenium and Iron in dried fruits and capsules agreed with the minimum values of daily intake (Araujo et al., 2013) while the rest were not quantified because they had concentration below the limit of detection (Sa et al., 2019).

Rocchetti and co-authors used ultra-high-pressure liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-ESI-QTOF-MS) combined with multivariate statistics to clarify the actual phenolic profiles and differentiate between 2 different kinds of goji berry – from China and Italy. (Rochetti et al., 2018). It was reported that flavonoids had the highest percentage with some few ones. Also, it was stated that the phenolic compounds of the Chinese Goji berries were better differentiated than the Italian ones. For the Italian berries, it was noted that flavones were the major compounds. These results showed similar outcomes when compared with other studies (Bondia-Pons, Savolainen, Törrönen, Martinez, Poutanen, & Hanhineva, 2014).

In another study, De Moura and coauthors used high performance liquid chromatography coupled with a photodiode array detector (HPLC-DAD) to determine the total phenolic compounds (TPC) as well as the best extraction conditions present in açaí, blueberry, and goji berry (de Moura et al., 2018). The highest and lowest TPC content found in each assay were as follows; in açaí, highest found at water treatment, lowest at ethanol treatment; in blueberry, highest and lowest at ethanol and water treatments; in goji berry, highest and lowest at water and ethanol treatments, respectively. It was reported that these differences were caused by huge differences of chemical compounds extracted based on the methodology used. Furthermore, it was stated that the extraction conditions were optimized – pure water and ethanol (800 g/L) both at extraction time and temperature 60min and 60^{o}C. Also, the solvent used for extracting goji berry, blueberry TPC and phenolic compounds were same as açaí. Mostly, this same result occurred with other fruits. This study was compared with Oldoni et al. (2015), the authors reported almost the same thing with ethanol (800g/L) being the best solvent but at slightly different temperature 70^{o}C and time 45 min of extraction (de Moura et al., 2018).

Ozkan and coauthors used liquid chromatography-mass spectrometry (LC-MS/MS) to determine the phenolic compounds present in water and methanolic extracts of Turkish goji berries. It was reported that the water extract was supplemented with phenolic acids, epicatechin, etc. While the methanolic extract was rich in flavonoids and anthocyanins – these were recognized for the first time in *L. barbarum* fruits. It was stated that when this work was compared with other works, Benchennouf et al., 2017 and Vulić et al., 2016, there were some discrepancies in the chemical composition of *L. barbarum* which may be due to soil content, climatic conditions, and cultivation zones. In all, methanolic extract has higher phenol and flavonoid content than the water content (Ozkan, Ozden, Toplan & Mat, 2018).

Mocan and his coauthors determined the main functional constituents of four goji berries cultivars which include the phenolic and flavonoid contents harvested in Italy. HPLC was used to analyze the phenolic and flavonoids content present in the berries. It was reported that Italian samples had the highest value for TPC, and Romania samples had the lowest values. Also, the highest value for total phenolic and flavonoid contents was seen at different samples (Mocan et al., 2019).

Tripodo and coauthors used pressurized liquid extraction (PLE) for the first time to extract phenolic compounds from four goji berries, using response surface methodology (RSM) for optimization of the PLE conditions, and high-pressure liquid chromatography- diode array detector tandem mass spectrometry (HPLC-DAD-MS/MS) for analysing the goji berry extracts. It was reported that the optimum PLE conditions for the total phenolic and flavonoid contents were obtained at 180^{o}C and 100% ethanolic extract. Furthermore, the traditional method of extraction - solid-liquid extraction (Protti et al., 2017) was compared with PLE method – new method. PLE method gave a higher yield which showed it was more effective than the conventional one. Then, the analysis of the goji berry extracts was done using HPLC-DAD-MS/MS at the PLE optimum conditions. In that study, a total of nine phenolic compounds with flavonols were identified from the four goji berry extracts (Tripodo, Ibanze, Cifuentes, Gilbert-Lopez, & Fanali, 2018).

Fatty-acid composition of goji berry species (*L. barbarum* and *L. chinensis* Mill.) grown in the Thessaly region of Greece was analyzed by GC-MS analysis and the

antioxidant activity was determined by Skenderidis and coauthors (Skenderidis et al., 2019). It was reported that polyunsaturated, saturated, and monounsaturated fatty acids (PUFA, SFA, MUFA) and more were found. Goji samples of *L. chinense* had the highest percentage while that of *L. barbarum* had the lowest. For the two species, linoleic acid was the dominant PUFA while from the imported ones, Mongolia sample had the lowest palmitic acid amongst the ones examined. Furthermore, it was stated that Oleic acid was the major MUFA as compared to other literature (Cossignani, Blasi, Simonetti, & Montesona, 2018) with similar results. Also, it was noted that PUFA were the most signified fatty acids (FA) as compared to other report by Blasi group which showed the same results (Blasi, Montesano, Simonetti, & Cossignani, 2017).

Furthermore, Skenderidis et al. (2019) determined the antioxidant activity of the two species with the imported one from Mongolia – *L. chinense* and *L. barbarum*. DPPH and $ABTS^{+}$ assays were measured and expressed as the IC_{50} value, and was stated by Zhen Chen, Bertin, R., & Froldi, G. (2013) as "the effective concentration of the samples required to obstruct 50% of the initial free radical". That is, the lower the IC_{50} value, the higher the antioxidant capacity. It was reported that fruits *of L. barbarum* had the higher antioxidant activity than that of fruits *of L. chinense* (IC_{50} value of *L. barbarum* were lower than *L. chinense*) while the Mongolia sample had the highest antioxidant activity (its IC_{50} value was the lowest) of all samples studied as compared to previous studies that showed similar results (Ionica, Nour, & Trandafir, 2012; Donno, Beccaro, Mellano, Ceritti, & Bounous, 2015; Skenderidis et al., 2017;Skenderidis et al., 2019).

Wojcieszek and coauthors determined the trace elements present in Goji berries. It was reported that these berries were grounded into fine powdery form and underwent digestion using a microwave. Then, the digested solutions were analysed using Inductively coupled plasma mass spectrometry (ICP-MS) and X-Ray fluorescence (XRF). It was stated that trace metal elements like Manganese, Iron, Copper, Zinc, Selenium, Molybdenum were found using ICP-MS, and others like Mercury, Arsenic, Chromium, and Silver were discovered using XRF. Though, Iron, Copper, and Zinc were also detected using XRF (Wojcieszek, Kwiatkowski, & Ruzik, 2017).

In a further study, Bertoldi and coauthors ascertained that there were 57 elements present in Italian and Asian goji berries. It was reported that Potassium was the most dominant macroelement in the two berries. Also, it was said that Italian goji berries had Copper, Molybdenum, Zinc and Selenium content twice more than Asian berries, but the latter had twice as much as the content of Sodium, Iron and Chromium in Italian berries. Furthermore, according to the Recommended Dietary Allowances (RDA), it was revealed that Copper and Molybdenum content were within the limits established (Bertoldi et al., 2019).

Pedro and coauthors determined the essential elements present in organic and conventional goji fruits. It was said that both were described by high amount of macroelements which include Sodium, Potassium, Phosphorus, Calcium, Magnesium and microelements which consist of Iron, Zinc, Copper, Manganese etc. The content from these elements were like the ones reported by Endes et al., (2015) and Llorent-Martínez et al., (2013). Furthermore, it was stated that organic fruits had higher contents of Potassium, Sodium, Calcium, Iron, Zinc and Copper elements than the conventional ones which could be due to application of fertilizers (Zhang et al., 2017). Also, the concentrations of heavy metals, cadmium, mercury and lead in organic goji berry were within the limits established compared to Mercury and Lead in conventional fruits which were above the maximum levels – 0.10mg/kg for both (Pedro et al., 2019).

1.4 Purpose of the Project

The focus is to determine the volatile profiles of Turkish goji berries by SPME-GC/MS, determination of phenolic compounds, elemental compositions, and total antioxidant capacity. This research is important because goji berry was just recently introduced to Turkey. So, there are not enough information about it yet. Regarding previous research, it was reported that goji berry extracts had unique beneficial effects in each country differently. Hence, conducting this research, it would give more information about the uniqueness of Turkish goji berries and would serve as a milestone for further studies.

CHAPTER TWO

EXPERIMENTAL PART

2.1 Chemicals and Materials

Sinapinic acid, trans-cinnamic acid p-coumaric acid, syringic acid, ferulic acid, and protocatechuic acid, were supplied from Alfa Aesar (Karlsruhe, Germany). Caffeic acid, gallic acid, chlorogenic acid, quercetin, vanillic acid, catechin, epicatechin, rutin, 2,2-diphenyl-1-picrylhydrazyl, alkane standards (C_6 to C_{30}), HPLC-grade methanol and acetonitrile were purchased from Sigma-Aldrich (St. Louis, USA). The standard stock solution containing 1000 mg L^{-1} of Mg, Ca, Fe, Cu, Mn, Zn, Pb, Hg, Cd, Se, Na, K, V and P in 2% nitric acid were obtained from Merck (Germany) and Perkin-Elmer (United Kingdom). All other reagents were analytical grade. Ultrapure water (18.2 M Ω cm) was used in the preparation of aqueous standard and sample solutions for elemental analysis.

The commercial SPME fibers (polyacrylate (PA), 85 µm thickness and divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 30 µm thickness), a manual SPME holder and a 20 mL of amber sampling vials including caps with PTFE septa were obtained from Supelco (Bellefonte, PA, USA). The commercial SPME fibers were preconditioned prior to SPME experiments according to the manufacturer's recommendation.

2.2 Samples

In this study, the genotypes belonging to *Lycium barbarum* (Hybrid GB and Natural GB) and *Lycium ruthenicum* (Black GB) goji berry were taken in September 2020 from the company of HZR Fidan in Aksaray province Kargın Village, Turkey as shown in Figure 2.1 below. Also, one goji berry (*Lycium barbarum*, GB from market) was purchased from a market in Izmir, Turkey. Hybrid GB, Natural GB and Black GB samples were dried in air at room temperature. Finally, all goji berry samples were prepared in powder form after drying using a freeze dryer.

Figure 2.1 Goji berry sample types (Personal archive, 2021)

2.3 ICP-OES Method

A Perkin Elmer Avio 200 model sequential inductively coupled plasma - optical emission spectrometer (ICP-OES) was used for element analysis in goji berry samples. The multi-element calibration solutions ranging from 25 to 100 µg L^{-1} for Cu, Pb, Mn, Zn, Cd, Hg, Se and V, 0.5 to 2 mg L^{-1} for P, 0.5 to 5 mg L^{-1} for Mg, Ca, 0.1 to 1 mg L^{-1} for Fe, 2.5 to 25 mg L^{-1} for K and 10 to 100 mg L^{-1} for Na were prepared using an individual stock solution of each element with Y internal standard addition. For determination below 25 µg L^{-1}, standard addition method was used. The dilution was applied to the samples whose measurement results were above the calibration range. The operational parameters were summarized in Table 2.1.

Table 2.1 Operational parameters for ICP-OES

Parameters	**Settings**
RF Power	1500 W
Plasma gas flow	8 L min^{-1}
Auxiliary gas flow	0.2 L min^{-1}
Nebulizer gas flow	0.65 L min^{-1}
Sample flow rate	1.00 mL min^{-1}
Flush time	35 sec
Spray Chamber	Cyclonic
Plasma view	Radial, Axial
Element (wavelength, nm)	Na (589.592), K (769.896), Mg (285.213), Ca (317.933)
	Fe (238.204), P (213.617), Cd (228.802), Cu (327.393)
	Mn (257.610), Zn (213.857), Pb (220.353), Se (196.026)
	V (292.464), Hg (253.652)
Correlation Coefficient	0.999
Number of Replicates	3
Internal standard (wavelength, nm)	Y (371.029)

2.4 HPLC Method

A Thermo Scientific Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific, Germany) with a diode array detector (DAD) was used in the determination of phenolic compounds. A C18 reversed-phase analytical column (15 cm × 4.6 mm × 5 µm ID, 100 Å) was used for separation of phenolic compounds (Figure 2.2). A MeOH:ACN: acetic acid 2% (v/v) solution (20:10:70 (v/v)) was used as a mobile phase and isocratic eltion was used in the analysis. The injection volume was 20 µL and the flow rate was 1 mL min^{-1}. FA, SnA, CA, CGA and pCA were detected at 320 nm, PrCA, R and VA at 254 nm, and SA, C, GA, EC and CnA at 278 nm.

A 1000 mg L^{-1} phenolic compound standard solution was prepared using MeOH. They were kept at 4 °C for further experiments. Working standard mix phenolic compound solutions were prepared from 1 to 20 µg mL^{-1} in methanol including 1.0 % (v/v) acetic acid. The obtained regression equations with coefficient of determination (R^2), detection wavelength and retention time were given in Table 2.2. The chromatgoraphic separation of standard phenolic compounds was given in Figure 2.3.

Figure 2.2 HPLC equipment (Personal archive, 2021)

Table 2.2 Analytical parameters of HPLC method

Phenolic compounds	λ (nm)	**t_R (min)**	**Regression equation**	**R^2**
Gallic Acid (GA)	278	2.92	y = 0.0509x+0.0132	0.9979
Protocatechuic Acid (PrCA)	254	3.13	y = 1.0989x-0.2759	0.9994
2,4-dihydrobenzoic acid (DBA)	254	3.35	y = 0.2234x-0.071	0.9985
Vanillic Acid (VA)	254	5.38	y = 0.5811x+0.4645	0.9935
Chlorogenic Acid (CGA)	320	3.31	y = 0.9299x+0.5260	0.9954
Cinnamic Acid (CnA)	278	4.31	y = 0.0352x+0.0132	0.9963
Syringic Acid (SA)	278	5.36	y = 0.0838x-0.0063	0.9991
Caffeic Acid (CA)	320	5.19	y = 1.6553x+1.1871	0.9969
P-Coumaric Acid pCA)	320	9.89	y = 1.4231x-0.3769	0.9999
Ferulic Acid (FA)	320	10.21	y = 0.7372x-0.0683	0.9977
Sinapinic Acid (SnA)	320	11.35	y = 0.1144x+0.0871	0.9954
Rutin ®	254	13.87	y = 0.1960x-0.0270	0.9972
Catechin (C)	278	9.16	y = 1.7628x-0.3168	0.9995
Epicatechin (EC)	278	13.82	y = 0.0876x-0.0426	0.9983

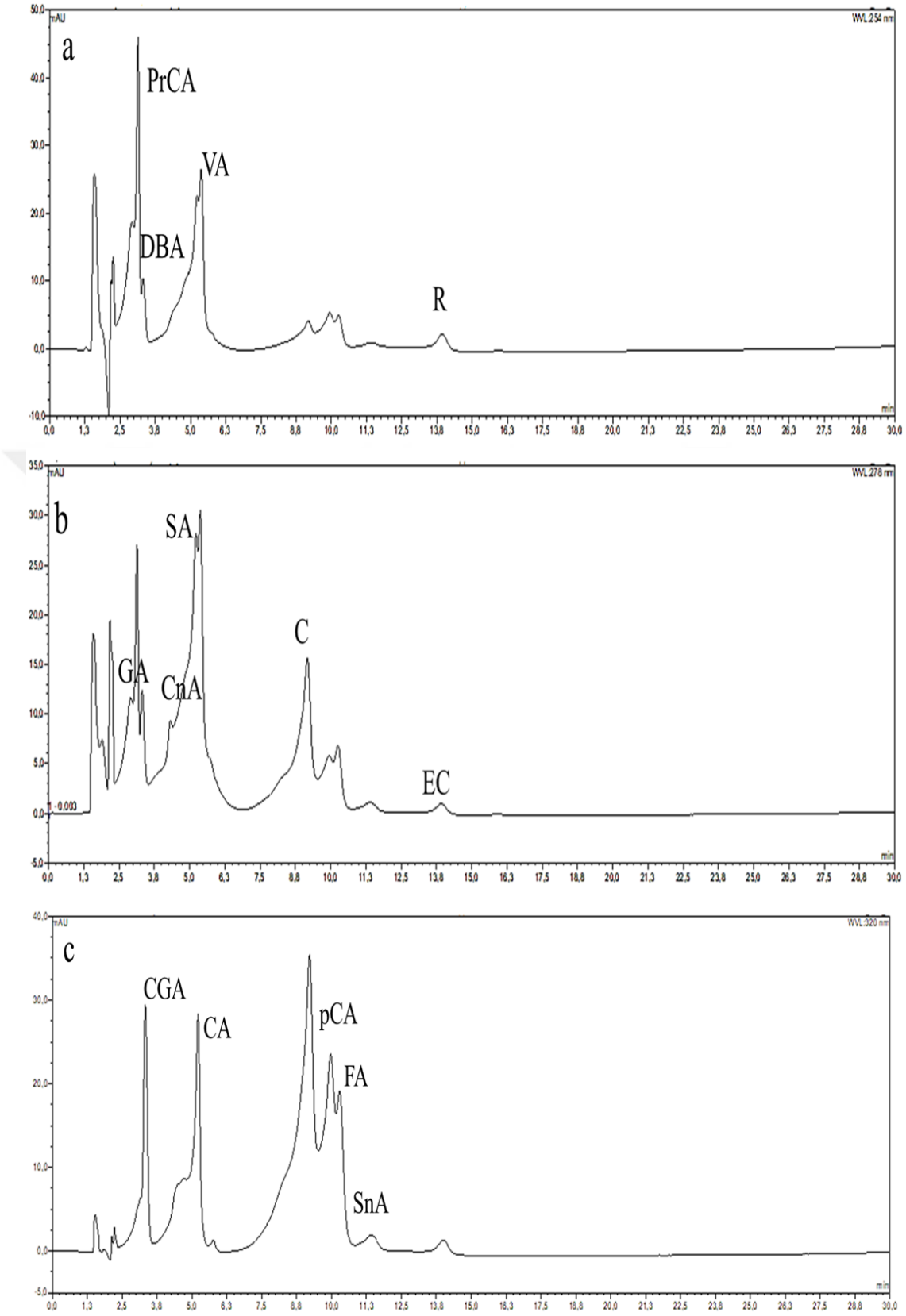

Figure 2.3 Chromatograms of phenolic compounds in HPLC at 254 nm(a), 278 nm (b) and 320 nm (c)

2.5 GC/MS Method

The optimization of the SPME method and analysis of volatile organic compounds in goji berry samples were performed using a Thermo Fisher Scientific Trace 1300 gas chromatography coupled with ISQ QD a single quadrupole mass spectrometer (West Palm Beach, FL, USA) (Figure 2.4). A TG-5MS capillary column (30 m x 0.25 mm, 0.25 μm film thickness) was used for chromatographic separation. The high purity grade helium as carrier gas was used at a flow rate of 1.2 mL min^{-1}. The injection was carried out at splitless mode. The ionization was done in the electron impact (EI) mode at 70 eV. The ion source temperatures and MS interface were set at 280 °C and 250 °C, respectively. The temperature program for separation of volatile organic compounds was at 50 °C for 3 min, then ramped to 180 °C at a rate of 3 °C min^{-1} and finally ramped to 280 °C at a rate of 20 °C min^{-1} (20 min hold). The mass scan range was 50-650 *m/z*. NIST 11 library and Adam's GC/MS library were used for mass spectral identification (Adams, 2009). The retention indices (RI) calculated using Van den Dool and Kratz equation (2.1) given

……..RI(x) = 100 Pz + 100 [(RT (x) - RT (Pz)) / (RT (Pz+1) - RT (Pz))]…..(2.1)

where RT: retention time as min, RT (P_z) ≤ RT(x) ≤ RT(P_{z+1}), and P_6 ... P_{30} are n-paraffins.

2.6 Antioxidant Activity With 2,2-diphenyl-1-picrylhydrazyl (DPPH) Method

DPPH radical was used to determine the free radical scavenging activity of goji berry samples according to the method of the Brand-Williams group (Brand-Williams, Cuvelier & Berset, 1995). Two grams of goji berry samples were extracted with 20 mL of methanol by ultrasonication at 60 °C for 2 h, then filtered and kept in the refrigerator up to analysis. Different volumes of methanol extract of goji berry samples were mixed with 250 μL of 1 mmol of methanolic DPPH solution and the total volume of the mixture was diluted to 1 mL using methanol. Later, the final solutions were kept in dark for 30 min, and then the solutions were measured at 517 nm using a double beam UV-Vis spectrophotometer in 3 parallel runs. The percentage of DPPH -

scavenging capacity of the goji berry extracts was calculated according to the following equation (2.2):

$$\%DPPH = ((Abs_{control} - Abs_{sample})/Abs_{control}) \times 100 \quad (2.2)$$

where $Abs_{control}$ and Abs_{sample} are the absorbance values of the control and the goji berry sample, respectively.

Figure 2.4 GC/MS equipment (Personal archive, 2021)

2.7 Sample Digestion and Multi-element Analysis

A 500 mg of goji berry sample was put into 50 mL teflon beaker, then 8 mL of concentrated HNO_3 was added, covered with a watch glass and kept overnight at room temperature. Later, 3 mL of 30% H_2O_2 was added, and the resulting mixture was heated on a hot plate at around 90 ^{0}C up to dryness (Figure 2.5). Finally, 5 mL of ultrapure water was added, the mixture was filtered and diluted to 50 mL with 0.1 M HNO_3 solution (Maghrabi, 2014). All experiments were performed in three parallels. The quantification of 14 macro, micro and trace elements was carried out by ICP-OES after digestion.

Figure 2.5 Digestion of samples (Personal archive, 2021)

2.8 Extraction Method for Phenolic Compound Analysis

In the extraction phenolic compounds from goji berry samples, a conventional solid-liquid extraction with methanol was used according to literature (Sa et al., 2017; Protti et al., 2017; Magiera, 2015). Briefly, a 500 mg of dried goji berry sample was extracted using 30 mL of methanol acidified with 0.1 mL of HCl by stirring on a shaker bath for 30 min. Then, the extracts were filtered, and the methanol was evaporated using rotary evaporator at 40 °C. The residue was dissolved in 1.5 mL of methanol, filtered through a PTFE 0.45 μm syringe filter before HPLC analysis. The extraction was repeated five times for each goji berry sample.

2.9 HS-SPME Method for Volatile Organic Compound Analysis

200 mg of dried goji berry sample was put into a 20 mL vial using a metallic block and heated for 30 min at 60 °C for incubation. Later, the SPME fiber was inserted into the vial and tip of the fiber was exposed to the headspace of the sampling vial at 60 °C for 60 min (Figure 2.6). Finally, the SPME fiber (PA or DVB/CAR/PDMS) was taken off and injected into the GC port. The goji berry samples extracted by the fiber were desorbed at 250°C for 5 min in splitless mode (Kaya et al., 2019).

Figure 2.6 Incubation of samples and insertion of the SPME fiber to the sampling vial (Personal archive, 2021)

2.10 Hydrodistillation Method for Volatile Organic Compound Analysis

To 500 mL round bottom flask, a 50 g of dried goji berry sample was put and then 250 mL water was added, the mixture was boiled for 8 h. After distillation, a 20 mL distillate was obtained and extracted with 20 mL of dichloromethane. Then, the separated organic phase was dried using anhydrous sodium sulfate. Later, the solvent was evaporated by rotary evaporation at 30 0C and the extract was transferred to a 1 mL vial using n-hexane and stored in a deep freezer for GC analysis (Figure 2.7).

Figure 2.7 Hydrodistillation and evaporation by rotary evaporator (Personal archive, 2021)

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Antioxidant Activity Based on DPPH

The antioxidant activities of the four goji berry sample types were determined by DPPH assay which measured each sample's ability in deactivating the radical species through electron transfer reactions. This was expressed as EC_{50} value as stated by Zhen Chen, Bertin, R., & Froldi, G. (2013) as "the effective concentration of the samples required to inhibit 50% of the initial free radical", that is, the lower the EC_{50} value, the higher the antioxidant capacity. The EC_{50} of DPPH radical-scavenging activity values of the samples, from *L . ruthenicum* (Black GB) to *L. barbarum* (GB from the market) ranged from 1332 to 9106 μg/mL (Table 3.1), with *L . ruthenicum* having the lowest EC_{50}. The percentage of DPPH-scavenging capacity of the goji berry methanolic extracts as shown in Figure 3.1 revealed that *L . ruthenicum* had the highest antioxidant capacity while *L. barbarum* (GB from the market) had the lowest.

According to Skenderidis et al, 2019, the IC_{50} (same as EC_{50}) values of different goji berry sample types in different seasons in 2016 ranged from 784 to 1254 μg/mL which is smaller compared to this study showed a similar trend in which the Mongolian GB type having the lowest IC_{50} value had the highest antioxidant capacity. Furthermore, in some other literature, the methanolic goji berry extracts showed higher antioxidant activity than the other type of extracts with significant differences among the goji berry genotypes EC_{50} values (Pires et al., 2018; Ozkan et al., 2018; Çolak et al., 2019). The differences between this study and others could be due to different goji berry genotypes, different geographical locations, and different seasons of collection of the goji berry samples.

Table 3.1 EC50 values of goji berry samples

Samples	**EC_{50}**
	(µg/mL)
Black GB	1332±26
Hybrid GB	3815±35
Natural GB	7627±52
GB from market	9106±78

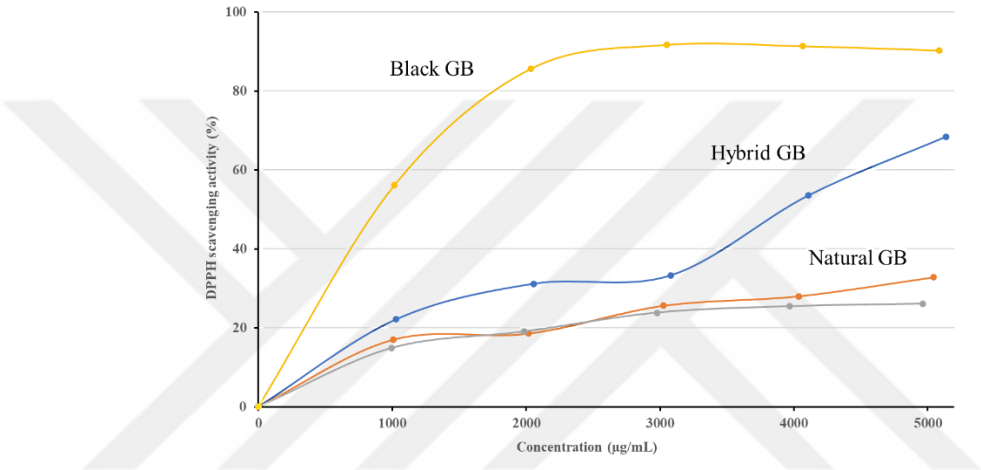

Figure 3.1 Antioxidant activity of goji berry methanol extract evaluated by DPPH scavenging activity

3.2 Elemental Composition

The four goji berry sample types were digested with HNO_3 and H_2O_2, each of these sample types was performed in three parallels followed by an analysis of elemental composition using ICP-OES. The quantification of elements is so vital due to their daily intake as a functional food (Hemp et al., 2016). Fourteen macro, micro, and trace elements were identified. The values attained for Calcium, Sodium, Magnesium, Phosphorus, and Potassium (Ca, Na Mg, P, K) macroelements were within the value range of 0.39 – 11.59 mg/g which were lower than those gotten from study by Llorent-Martínez et al., (2013); Skowron et al., (2017). According to Vigo, Narita, & Marques, (2004), it was said that "this could be due to mineral variations in the soil, as well as lower bioaccumulation of these minerals in the fruit". There were more macroelements in *L. ruthenicum* followed by *L. barbarum* (Hybrid GB) compared to other goji berry

types. The P content in *L. barbarum* (Hybrid GB) which was higher than *L. barbarum* (Natural GB) in this study showed similar result to the work reported by Oğuz et al., (2019). K is the most abundant macroelement in this study which is the same as other literatures (Bertoldi et al., 2019; Pedro et al., 2019). The K content in *L. barbarum* (Hybrid GB) is higher than *L. barbarum* (Natural GB) in this study compared to the one reported by Oğuz et al., (2019) where the *L. barbarum* (Natural GB) was slightly higher. However, the *L. barbarum* (Natural GB) is higher than *L. barbarum* (Hybrid GB) in this study compared to the work reported by the same authors where *L. barbarum* (Natural GB) was slightly lower. In addition, the Mg content showed a similar result as K content in this study compared the same authors where the *L. barbarum* (Natural GB) was higher. Na concentration was higher than P as already reported by Llorent-Martínez et al., (2013).

The values gotten for essential microelements, Se, Zn, Mn, Cu, and Fe ranged from 0.82 – 104.52 µg/g. Also, there were more microelements in *L. ruthenicum* followed by *L. barbarum* (GB from the market) in this study. Fe is the most abundant microelement in this study as reported by Oğuz et al., (2019). Furthermore, the Fe content in *L. barbarum* (Natural GB) is higher than *L. barbarum* (Hybrid GB) as reported by Oğuz et al., (2019). In addition, Cu content in *L. barbarum* (Hybrid GB) is higher than *L. barbarum* (Natural GB) as reported by the same authors. However, the Mn and Zn contents in *L. barbarum* (Natural GB) were lower than *L. barbarum* (Hybrid GB) in this study compared to the study reported by the same authors where *L. barbarum* (Natural GB) was higher. Mn concentration was higher than Cu as reported by Llorent-Martínez et al., (2013). The attained values for Iron, Manganese, and Zinc (Fe, Mn, and Zn) were higher than those inferred by Yang and Kang (2017) who carried out the analysis by ICP-MS. According to Rodrigues et al., (2019) it was said that "this could be due to differences in geographical changes in related soil type, climate and cultivation conditions which could lead to changes in the mineral content of the samples studied, such as conditions of storage and transport of these samples". Furthermore, there were some concentrations of trace elements, V (<0.49µg/g), Pb, Hg, and Cd (<2.42µg/g) that were identified.

Table 3.2 Total concentration of various elements in goji berry samples

Elements	Units	Black GB	Hybrid GB	Natural GB	GB from market
Na	(mg/g)	2.52±0.29	3.55±0.28	1.61±0.30	3.00±0.27
K	(mg/g)	11.59±1.54	10.82±1.28	10.21±1.38	10.19±0.47
Mg	(mg/g)	1.04±0.18	0.83±0.12	0.60±0.04	0.68±0.07
Ca	(mg/g)	2.01±0.07	0.39±0.01	0.44±0.02	0.85±0.03
P	(mg/g)	1.64±0.02	1.69±0.03	1.67±0.04	1.84±0.06
Fe	(µg/g)	104.52±12.60	30.38±4.91	38.76±4.38	66.09±2.15
Cu	(µg/g)	<2.45	9.35±0.64	6.31±0.38	6.67±0.86
Mn	(µg/g)	10.11±1.60	10.00±1.90	7.94±0.42	11.13±1.56
Zn	(µg/g)	15.06±0.23	29.79±2.05	27.61±1.50	27.78±1.37
Se	(µg/g)	0.82±0.01	1.10±0.02	0.90±0.01	1.08±0.03
V	(µg/g)	<0.49	<0.49	<0.49	<0.49
Pb	(µg/g)	<2.42	<2.42	<2.42	<2.42
Hg	(µg/g)	<2.42	<2.42	<2.42	<2.42
Cd	(µg/g)	<2.42	<2.42	<2.42	<2.42

3.3 Phenolic Compounds Profile

According to Haminiuk et al., (2012), it was reported that "phenolic compounds can be divided into two main groups: phenolic acids (hydroxylated derivatives of benzoic and cinnamic acids) and flavonoids, including flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones". The analysis of the phenolic compounds by using HPLC in each of the four goji berry extracts was performed in different wavelengths from 254, 278, and 320 nm (Figures 3.2 – 3.5). Retention times, wavelengths and regression equation data of compounds are given in Table 2.2. 12 phenolic compounds were identified in the goji berry samples: 4 hydroxybenzoic acids (gallic acid, procatechuic acid, vanillic acid, syringic acid), 5 hydroxycinnamic acids (p-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, sinapinic acid), and 3 flavonoids (catechin, epicatechin acid, rutin). All the 12 phenolic compounds were found in *L. ruthenicum* and *L. barbarum* (GB from the market). Chlorogenic acid was

not found only in *L. barbarum* (Hybrid GB) and *L. barbarum* (Natural GB), while gallic acid, vanillic acid, and syringic acid were not identified only in *L. barbarum* (Hybrid GB). Among the 12 compounds, rutin has the largest mass fraction as shown in another literature by Carvalho et al., (2016).

Similar results were reported by De Moura et al., (2018) where three phenolic acids (caffeic acid, p-coumaric acid, ferulic acid) in concentrations 0.151mg/g, 0.123mg/g, 0.069mg/g, and two flavonoids (catechin, rutin) in concentrations 0.050 mg/g and 0.326 mg/g were found in *L. barbarum* extracts. In this study, the concentrations of caffeic acid, p-coumaric acid, ferulic acid ranged from 0.005 – 0.254mg/g, 0.004 – 0.008mg/g, 0.001- 0.238mg/g, and (catechin, rutin) ranged from 0.015 – 0.135mg/g and 0.134 – 0.672mg/g. The values reported by De Moura and coauthors were within the values revealed in this study except p-coumaric which was lower. According to De Moura et al., (2018) it was inferred that "this could be as a result of the method used in extraction, equipment, identification and separation conditions being different". Also, Donno et al. (2015) found the same 3 phenolic acids, and epicatechin acid in *L. barbarum* from Italy. Wang et al. (2010) in their studies found 2 of the same compounds – rutin and caffeic acid with a different one - quercetin. In addition, all the phenolic compounds found in this study were identified in the *L. barbarum* extracts of another work by Carvalho et al., (2016).

Table 3.3 Concentration of phenolic compounds in goji berry samples

Samples	Black GB (µg/g)	Hybrid GB (µg/g)	Natural GB (µg/g)	GB from market (µg/g)
GA	109±2		76.26±3.44	268±9
PrCA	11.55±0.68	8.59±0.47	10.02±0.84	4.61±0.92
VA	17.9±1.4		3.48±0.06	39.26±1.42
SnA	240±2	53.2±1.52	45.48±2.14	51.48±1.83
CGA	466±6			201±3
SA	10.61±0.74		5.27±0.62	29.98±1.74
pCA	8.47±0.36	4.02±0.24	5.43±0.08	5.71±0.56
FA	238±2	3.42±0.34	1.24±0.05	6.82±0.34
R	672±5	134±2	198±3	246±5
CA	254±6	49.1±2.36	5.27±0.64	152±6
C	15.83±0.92	132±3	120±2	135±5
EC	38.48±2.32	146±3	308±7	51.84±2.66

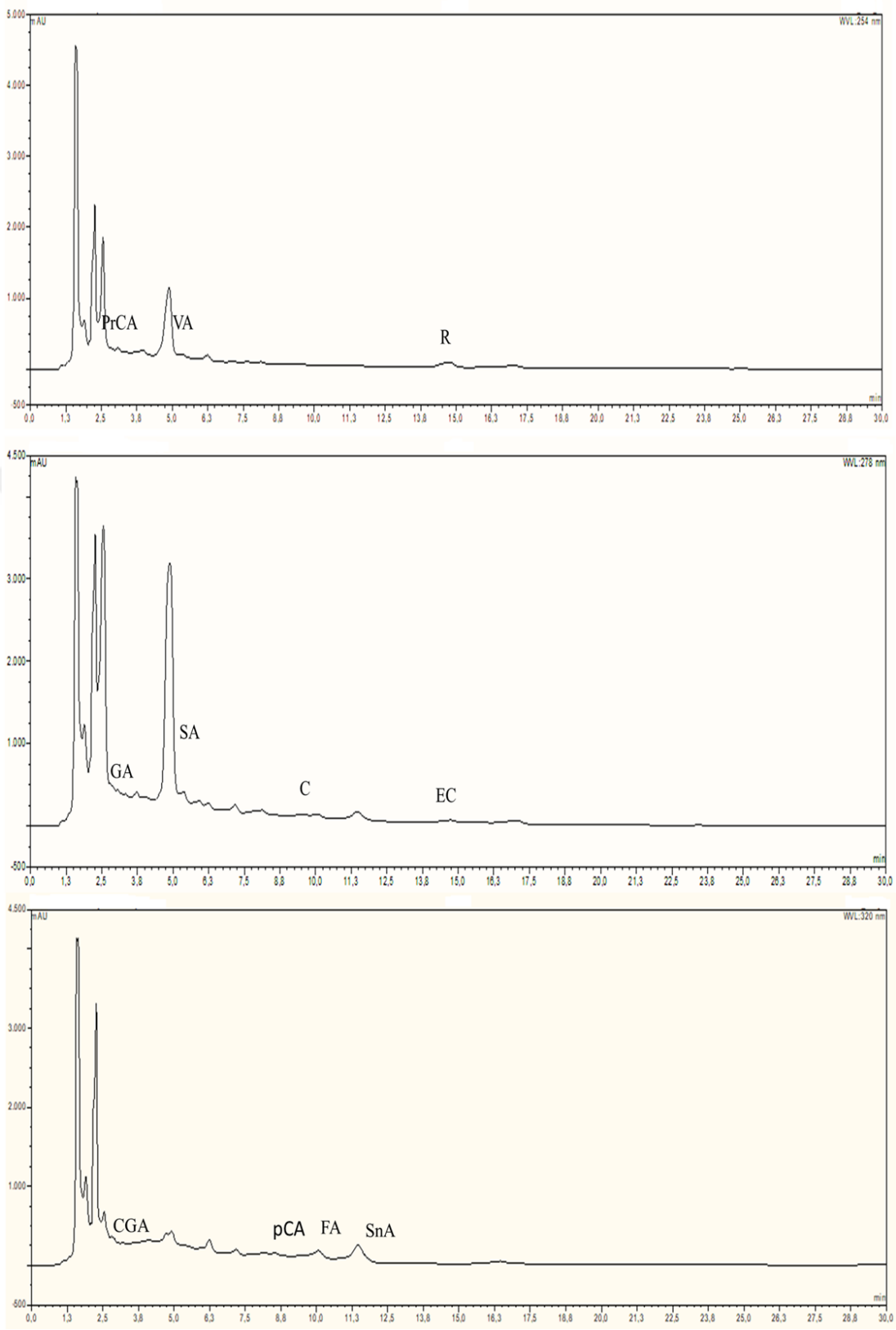

Figure 3.2 Chromatograms obtained for phenolic compounds in Black GB sample at 254 nm, 278 nm and 320 nm

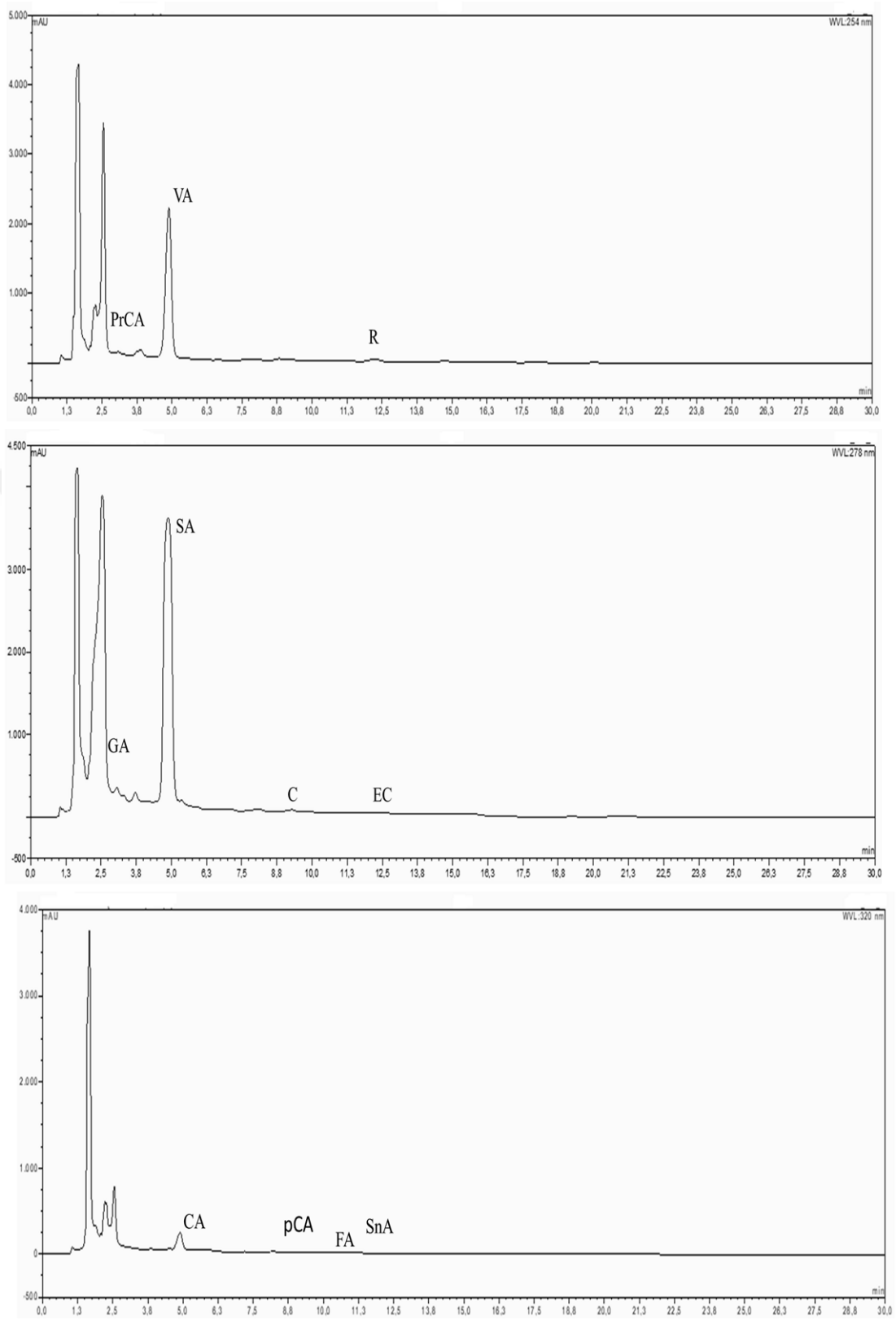

Figure 3.3 Chromatograms obtained for phenolic compounds in Hybrid GB sample at 254 nm, 278 nm and 320 nm

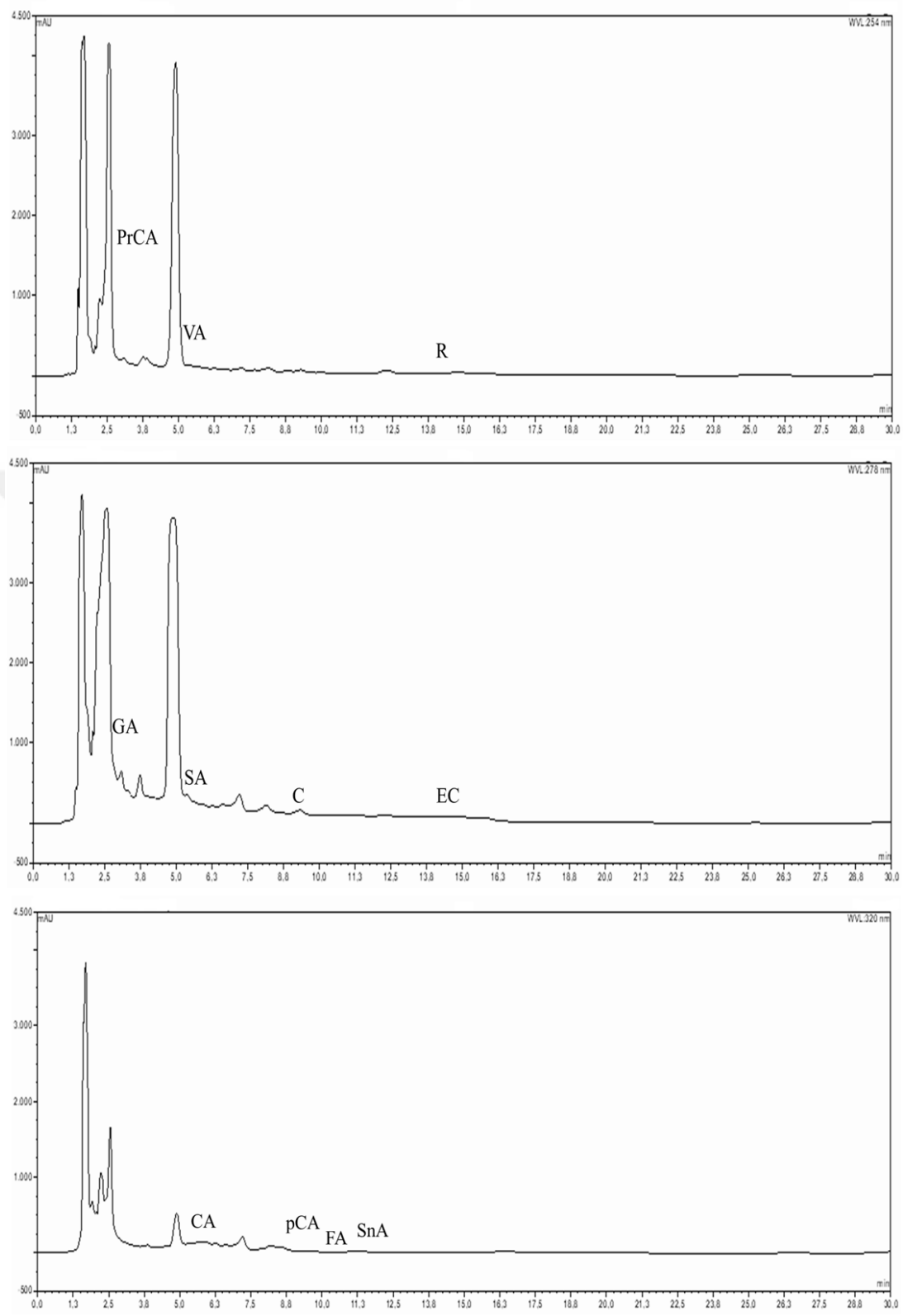

Figure 3.4 Chromatograms obtained for phenolic compounds in Natural GB sample at 254 nm, 278 nm and 320 nm

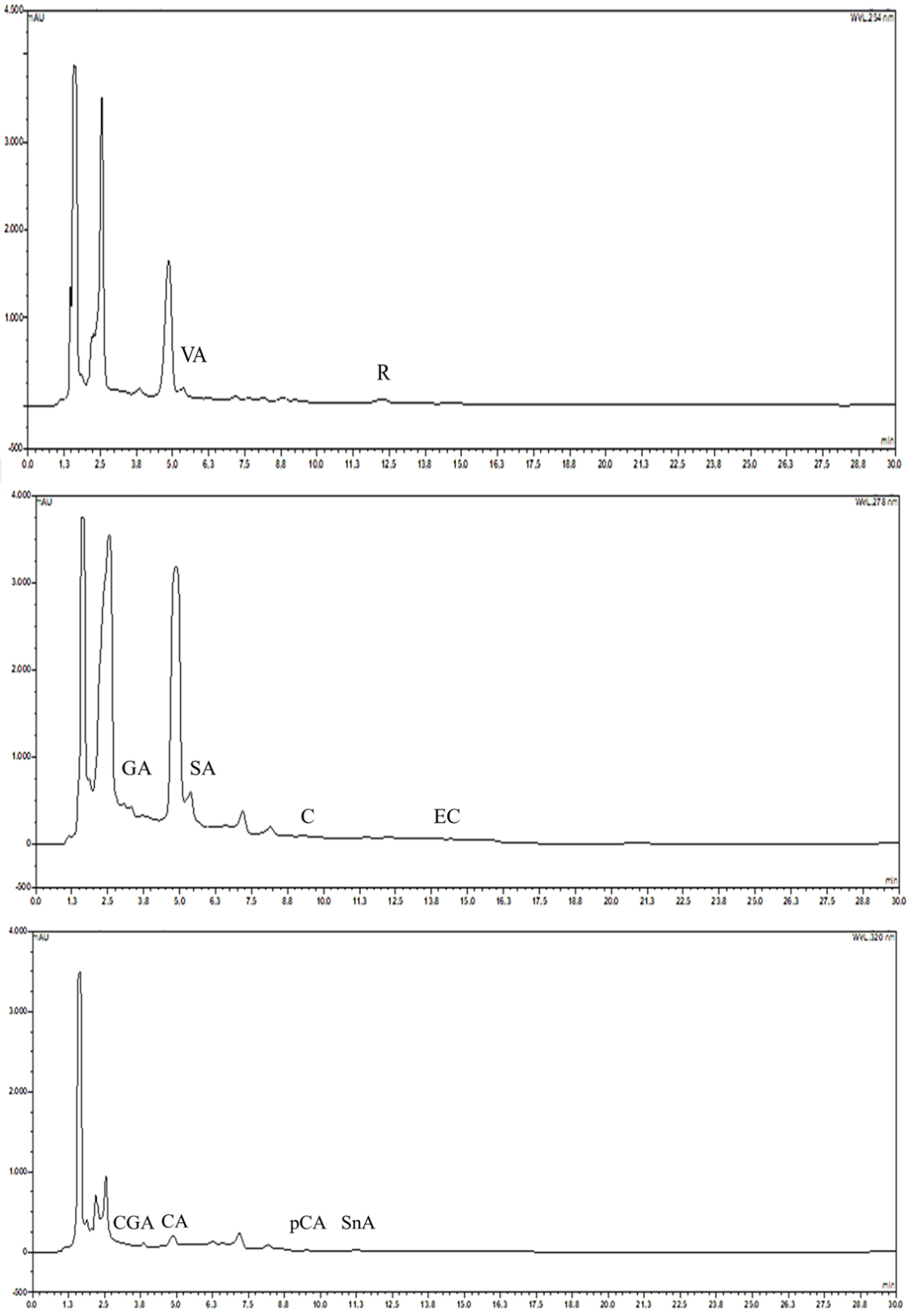

Figure 3.5 Chromatograms obtained for phenolic compounds in GB from market sample at 254 nm, 278 nm and 320 nm

3.4 Identification of Volatile Compounds in Goji Berry

The HS-SPME method with commercial SPME fibers (DVB/CAR/PDMS, PA) and HD method revealed that there were more volatile compounds found in *L. ruthenicum* and *L. barbarum* (Hybrid GB) using DVB/CAR/PDMS fiber compared to the other *L. ruthenicum* and *L. barbarum* (Hybrid GB) using PA fiber and HD. Also, the volatile compounds identified in *L. barbarum* (Natural GB) and *L. barbarum* (Commercial GB) using PA fiber were more than the ones using DVB/CAR/PDMS fiber and HD. However, the numbers were closer with DVB/CAR/PDMS fiber. In this study, 96 volatile compounds in the goji berries were identified by NIST 11 library and Adam's GC/MS library (Adams, 2007) from the 188 volatiles detected. These include 17 aldehydes, 12 alcohols, 21 esters, 18 ketones, 1 terpene, 2 furans, 10 fatty acids, 1 aromatic carboxylic acid, 1 aromatic compound, 6 alkanes, 1 alkene, 1 pyrazine, 1 pyrrole, 1 naphthalene, and 3 miscellaneous compounds via SPME method using DVB/CAR/PDMS, PA fibers and HD (Table 3.4 – 3.6). Similar outcomes were reported by J. Lu et al., (2009), out of the 193 volatiles detected in *L. barbarum* L., only 56 volatile compounds were identified which include aldehydes, alcohols, esters, ketones, furans, etc. It was revealed that some of the volatile compounds such as (E)-2-hexenal, nonanal, 1-octen-3-ol, linalool, etc which were important volatiles in Ningxia goji berries (Lu et al., 2009) were also found in this study. In addition, hexadecenoic acid, myristic acid, ethyl hexadecanoate, ethyl linoleate and others were found in *L. barbarum* and *L. ruthenicum* by Altintas and coworker (2006). Thisvolatiles were also determined in this study.

Furthermore, Kim et al., (2009) identified 130 volatile components from *Lycium chinensis* Miller, including acids, alcohols, aldehydes, aromatics, esters, ketones, terpenes. Also, similar results reported by Chen et al., (2015) identified 57 volatile compounds by HS-trap-GC-MS from *Lycium barbarum* L., which include aliphatic alcohol, aliphatic aldehydes, aliphatic ketones, furan, and aliphatic hydrocarbon. The major fatty acids found by Zhao, (2018) include linoleic acid, oleic acid, palmitic acid, and stearic acid were found in this study as well. However, the differences in this study and other literatures could be due to different goji berry sample types, different geographical locations, and different seasons of collection of the goji berry samples.

Lastly, more volatile compounds were identified using the SPME fibers (DVB/CAR/PDMS and PA) compared to the HD method. The chromatograms obtained for volatile organic compounds in GB samples with HS-SPME method using DVB/CAR/PDMS, PA fibers and HD method is shown in Figures 3.6 – 3.8 below.

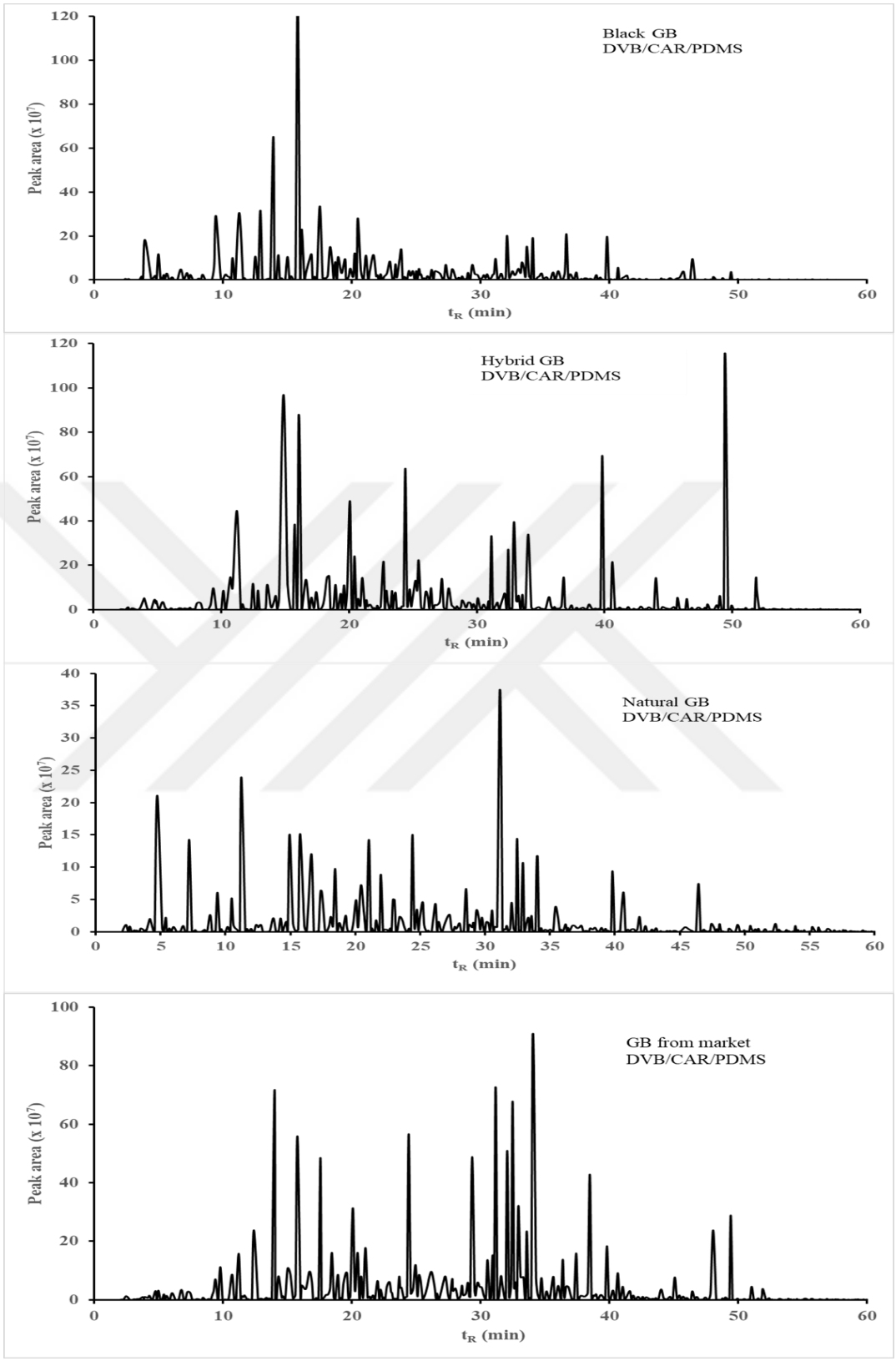

Figure 3.6 GC total ion chromatograms obtained for volatile organic compounds in GB samples with HS-SPME method using DVB/CAR/PDMS fiber

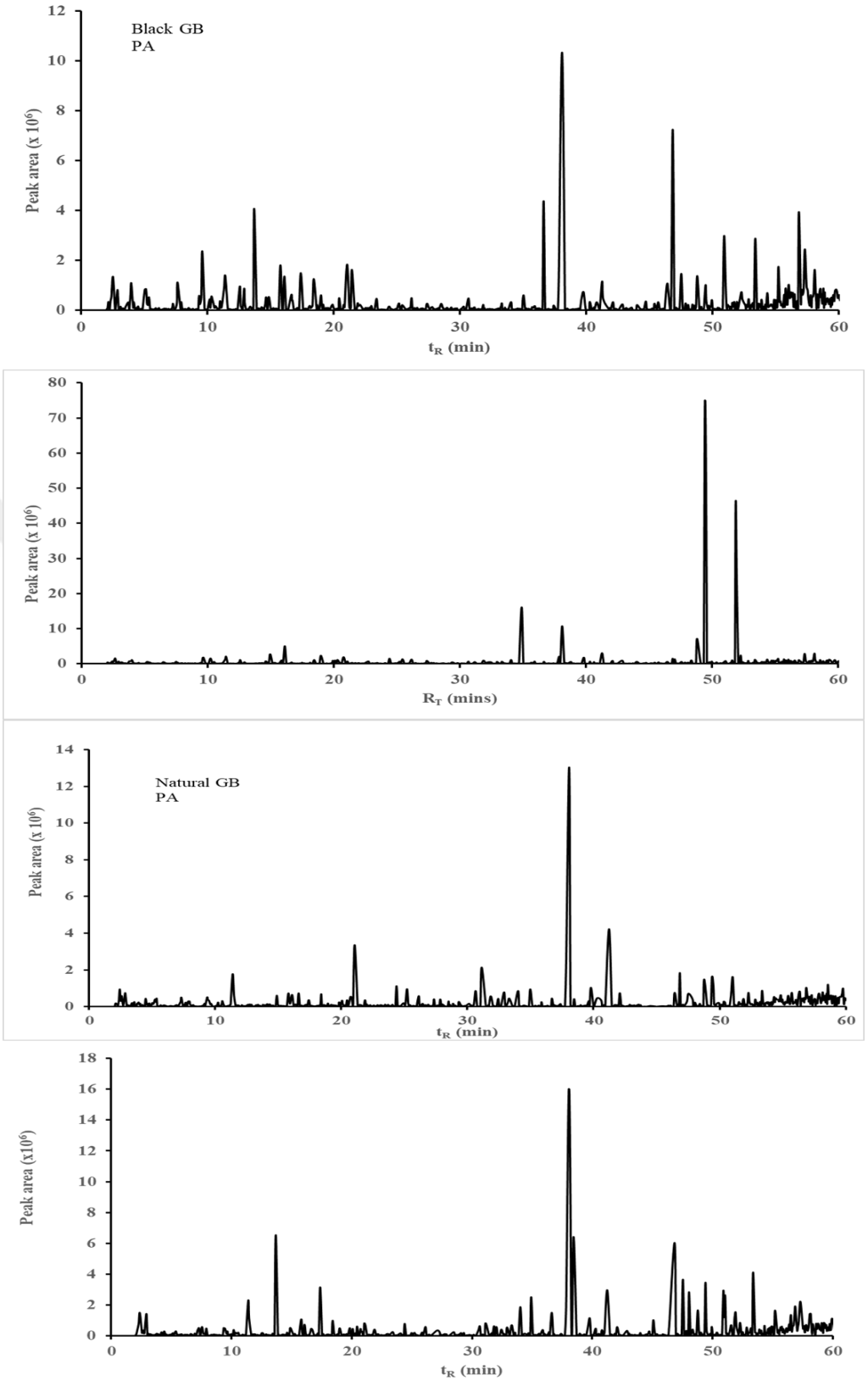

Figure 3.7 GC total ion chromatograms obtained for volatile organic compounds in GB samples with HS-SPME method using PA fiber

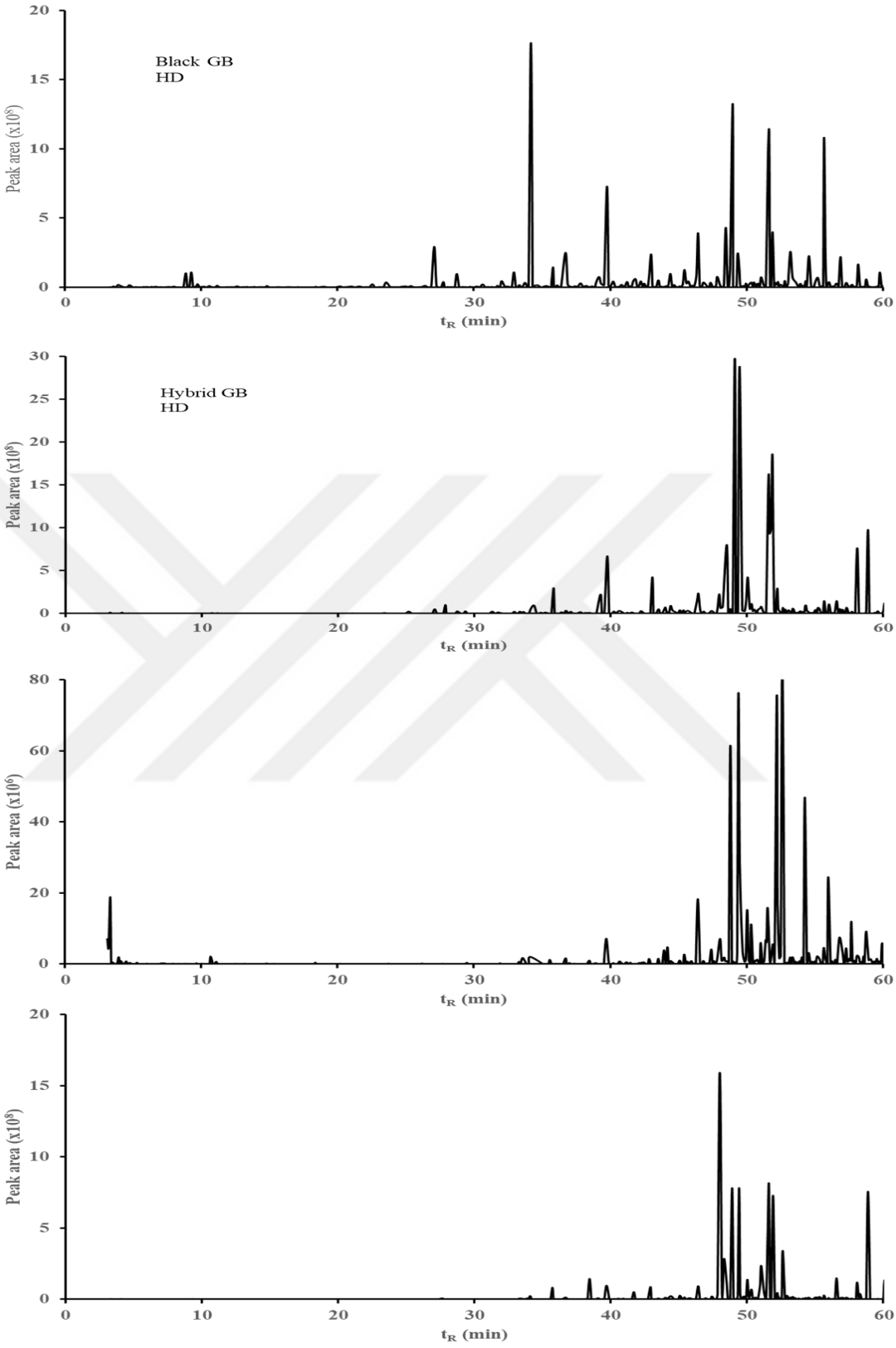

Figure 3.8 GC total ion chromatograms obtained for volatile organic compounds in GB samples with HD method

Table 3.4 Identified volatile compounds with DVB/CAR/PDMS fiber in goji berry samples

	Area, % by GC/MS (x±s)							
Compounds	**Formula**	**t_R (min)**	**RI_{lit}**	**RI_{calc}**	**Black GB**	**Hybrid GB**	**Natural GB**	**GB from market**
Heptane	C_7H_{16}	3.14	700	702				
Isoamylol	$C_5H_{12}O$	3.66	731	732				
1-Pentanol	$C_5H_{12}O$	4.23	762	765		0.102 ± 0.01	0.601 ± 0.082	0.150±0.026
2,6-dimethyl-3-Heptene	C_9H_{18}	5.21	815	816				
Furfural	$C_5H_4O_2$	5.59	828	832	0.366 ± 0.021	0.173 ± 0.043	0.31 ± 0.023	0.139±0.059
Methyl tiglate	$C_6H_{10}O_2$	6.4	863	764				
Styrene	C_8H_8	6.75	880	879	0.449 ± 0.049	0.124 ± 0.051	0.237 ± 0.023	0.279±0.072
(Z)-4-Heptenal	$C_7H_{12}O$	7.1	893	893	0.23 ± 0.016	0.044 ± 0.018		
Heptanal	$C_7H_{14}O$	7.32	901	901				
Butyrolactone	$C_4H_6O_2$	7.6	908	908	0.324 ± 0.045	0.202 ± 0.045		0.193±0.086
2,5-Hexanedione	$C_6H_{10}O_2$	8.02	920	919	0.092 ± 0.011	0.298 ± 0.046		
2-propenyl 3-Methylbutanoate	$C_8H_{14}O_2$	8.89	931	941		0.255 ± 0.035	0.829 ± 0.052	
(2E)-Heptenal	$C_7H_{12}O$	9.11	947	949	0.1 ± 0.018	0.185 ± 0.024		0.098±0.021
Benzaldehyde	C_7H_6O	9.35	952	953	0.881 ± 0.048	0.998 ± 0.155	1.414 ± 0.226	1.033±0.034
Hexanoic acid	$C_6H_{12}O_2$	9.89	967	966	0.178 ± 0.018		0.119 ± 0.025	0.128±0.080
1-Octen-3-one	$C_8H_{14}O$	10.14	972	973	0.129 ± 0.012			
1-Octen-3-o1	$C_8H_{16}O$	10.18	974	974	0.395 ± 0.057	0.864 ± 0.17	0.199 ± 0.012	
2-Pentylfuran	$C_9H_{14}O$	10.71	984	987	1.289 ± 0.084	1.31 ± 0.075	0.567 ± 0.075	1.283±0.024
Benzyl alcohol	C_7H_8O	12.47	1026	1028	1.337 ± 0.125	1.008 ± 0.057	0.571 ± 0.012	
Benzeneacetaldehyde	C_8H_8O	12.86	1036	1036	3.763 ± 0.234	0.706 ± 0.035	0.322 ± 0.054	0.060±0.029
(E)-2-Octen-1-al	$C_8H_{14}O$	13.41	1049	1049	0.11 ± 0.032	1.11 ± 0.06		0.069±0.001
2-Acetylpyrrole	C_6H_7NO	13.69	1054	1055	7.887 ± 0.258	0.103 ± 0.01	0.35 ± 0.092	
1-Octanol	$C_8H_{18}O$	14.29	1063	1067	1.517 ± 0.195	0.491 ± 0.016	0.495 ± 0.058	0.543±0.045

Table 3.4 continues

tetramethyl-Pyrazine	$C_8H_{12}N_2$	14.84	1083	1080	1,414 ± 0,236	7,457 ± 0,49	3,667 ± 0,415	1,060±0,121
2-Nonanone	$C_9H_{18}O$	15.2	1087	1088	0,175 ± 0,056	0,99 ± 0,092	0,326 ± 0,019	0,622±0,047
Nonanal	$C_9H_{18}O$	15.77	1100	1100	15,889 ± 1,48	3,025 ± 0,256	3,45 ± 0,604	4,218±0,281
Benzeneethanol	$C_8H_{10}O$	16.06	1107	1107	2,797 ± 0,656	7,45 ± 0,253	0,261 ± 0,037	0,427±0,050
Methyl octanoate	$C_9H_{18}O_2$	16.8	1123	112	2,719 ± 0,112	1,353 ± 0,143	2,902 ± 0,603	1,340±0,085
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	$C_6H_8O_4$	17.37	1134	1135	4,639 ± 0,45	0,921 ± 0,079	3,233 ± 0,118	5,839±0,113
Menthone	$C_{10}H_{18}O$	18.03	1148	1149	0,395 ± 0,034	0,649 ± 0,089	0,73 ± 0,01	0,361±0,058
(E)-2-Nonenal	$C_9H_{16}O$	18.29	1157	1155	1,776 ± 0,223	1,179 ± 0,097	0,223 ± 0,026	0,195±0,026
1-Nonanol	$C_9H_{20}O$	18.94	1169	1169	1,267 ± 0,144	0,74 ± 0,093	0,233 ± 0,065	0,616±0,081
Naphthalene	$C_{10}H_8$	19.15	1178	1175	0,386 ± 0,023	0,062 ± 0,01	1,004 ± 0,23	0,072±0,016
Benzoic acid	$C_7H_6O_2$	19.36	1178	1178	1,288 ± 0,06	0,601 ± 0,025	0,549 ± 0,02	0,845±0,040
Methyl salicylate	$C_8H_8O_3$	19.88	1190	1189	0,846 ± 0,024	1,066 ± 0,078	0,411 ± 0,038	0,943±0,048
Safranal	$C_{10}H_{14}O$	20.05	1197	1193	0,196 ± 0,012	3,798 ± 0,252	1,254 ± 0,12	2,386±0,376
Dodecane	$C_{12}H_{26}$	20.22	1200	1197	1,427 ± 0,066		0,13 ± 0,012	0,218±0,033
Decanal	$C_{10}H_{20}O$	20.43	1201	1201	2,879 ± 0,081	1,352 ± 0,64	1,571 ± 0,108	1,197±0,356
β-Cyclocitral	C_8H_8O	21.05	1217	1215	1,378 ± 0,111	1,101 ± 0,133	4,088 ± 0,467	1,507±0,104
5-Hydroxymethylfurfural	$C_6H_6O_3$	21.41	1224	1223	0,623 ± 0,047	0,436 ± 0,073	0,091 ± 0,076	0,241±0,018
Methylethylmaleimide	$C_7H_9NO_2$	21.61	1235	1227	1,497 ± 0,098	0,202 ± 0,034	0,378 ± 0,053	0,234±0,019
2-Phenylethyl acetate	$C_{10}H_{12}O_2$	22.67	1254	1251	0,466 ± 0,016	0,105 ± 0,01	0,08 ± 0,008	0,080±0,016
Nonanoic acid	C9H18O2	23.5	1267	1269	3,175 ± 0,093	0,948 ± 0,098	1,706 ± 0,076	1,878±0,208
2-Undecanone	$C_{11}H_{22}O$	24.39	1293	1289	0,48 ± 0,018	5,453 ± 0,25	3,683 ± 0,152	4,309±0,219
Tridecane	$C_{13}H_{28}$	24.7	1300	1296	0,493 ± 0,023	0,795 ± 0,054	0,849 ± 0,045	0,628±0,110
2-Undecanol	$C_{11}H_{24}O$	24.93	1303	1301	0,426 ± 0,014	0,332 ± 0,06	0,439 ± 0,068	0,880±0,073
4-Vinylguaiacol	$C_9H_{10}O_2$	25.19	1309	1307	0,715 ± 0,032	1,103 ± 0,13	1,337 ± 0,083	0,808±0,045
Limonene aldehyde	$C_{11}H_{18}O$	26.02	1328	1327	0,354 ± 0,017	0,739 ± 0,054	0,614 ± 0,052	0,855±0,010
Eugenol	$C_{10}H_{12}O_2$	27.09	1356	1352	0,047 ± 0,011	0,422 ± 0,068	0,463 ± 0,144	0,812±0,114

Table 3.4 continues

Nonan-1,4-olide	$C_9H_{16}O_2$	27.26	1358	1356	0.882 ± 0.053	1.134 ± 0.068	0.595 ± 0.086	0.655±0.041
Decanoic acid	$C_{10}H_{20}O_2$	27.77	1364	1368	1.393 ± 0.051	1.008 ± 0.124	0.875 ± 0.027	1.298±0.116
β-(E)-Damascenone	$C_{13}H_{18}O$	28.26	1383	1379	0.384 ± 0.021	0.287 ± 0.048	0.164 ± 0.002	0.438±0.009
Vanillin	$C_8H_8O_3$	28.78	1393	1391	0.102 ± 0.011	0.325 ± 0.047	0.34 ± 0.12	0.224±0.045
Decanoic acid. ethyl ester	$C_{12}H_{24}O_2$	28.84	1392	1392	0.569 ± 0.025	0.308 ± 0.012	1.33 ± 0.09	2.021±0.208
Tetradecane	$C_{14}H_{30}$	29.05	1400	1397	0.21 ± 0.014	0.489 ± 0.063	0.366 ± 0.025	0.683±0.059
Dodecanal	$C_{12}H_{24}O$	29.3	1408	1404		0.198 ± 0.054	0.853 ± 0.011	1.030±0.123
α-Ionone	$C_{13}H_{20}O$	30.09	1426	1423	1.823 ± 0.0241	2.884 ± 0.187	9.278 ± 0.155	6.826±0.117
Dihydro-β-ionone	$C_{13}H_{22}O$	30.57	1434	1434	1.494 ± 0.156	0.527 ± 0.025	1.997 ± 0.231	3.400±0.452
Geranylacetone	$C_{13}H_{22}O$	31.15	1453	1448	0.325 ± 0.024			0.462±0.050
1-Dodecanol	$C_{12}H_{26}O$	32	1469	1469	0.616 ± 0.011	0.134 ± 0.01	0.081 ± 0.011	0.825±0.079
3,4-Dehydro-β-ionone	$C_{13}H_{18}O$	32.39	1485	1479	0.623 ± 0.024	3.287 ± 0.12	2.883 ± 0.317	2.507±0.220
5-Methyl-2-phenyl-2-hexenal	$C_{13}H_{16}O$	32.63	1485	1485	0.377 ± 0.013	0.655 ± 0.058	0.35 ± 0.01	0.556±0.050
2-Tridecanone	$C_{13}H_{26}O$	32.9	1495	1491	1.574 ± 0.12	0.605 ± 0.04	0.726 ± 0.032	0.555±0.085
Pentadecane	$C_{15}H_{32}$	33.07	1500	1496	2.283 ± 0.187	2.893 ± 0.207	3.81 ± 0.691	7.775±0.329
Valencene	$C_{15}H_{24}$	33.21	1496	1499	0.624 ± 0.065	0.769 ± 0.188	0.653 ± 0.221	0.72±0.063
Dihydroactinidiolide	$C_{11}H_{16}O_2$	34.02	1525	1521	2.572 ± 0.245	0.233 ± 0.027	0.317 ± 0.165	0.467±0.027
Dodecanoic acid	$C_{12}H_{24}O_2$	35.7	1565	1563	0.34 ± 0.023	1.408 ± 0.189	0.22 ± 0.031	0.320±0.017
Diethyl phthalate (plastizer. comtaminant)	$C_{12}H_{14}O_4$	36.69	1590	1588	0.416 ± 0.06	0.208 ± 0.012	0.483 ± 0.083	0.692±0.056
Ethyl laurate	$C_{14}H_{28}O_2$	36.76	1594	1591		± 0.012		
Tetradecanal	$C_{14}H_{28}O$	37.41	1611	1607	0.093 ± 0.013	0.08 ± 0.011	0.079 ± 0.012	0.221±0.015
Benzophenone	$C_{13}H_{10}O$	37.81	1621	1618	0.226 ± 0.017	0.066 ± 0.01	0.32 ± 0.032	0.258±0.027
(1-butylheptyl)-Benzene	$C_{17}H_{28}$	38.27	1633	1631	1.769 ± 0.154	7.187 ± 0.252		2.523±0.139
3-Keto-β-ionone	$C_{13}H_{18}O_2$	39.48	1661	1663	0.768 ± 0.135	1.935 ± 0.139	1.766 ± 0.25	0.700±0.066
1-Tetradecanol	$C_{14}H_{30}O$	39.78	1671	1672	0.384 ± 0.021	0.287 ± 0.048	0.164 ± 0.002	0.438±0.009
2-Pentadecanone	$C_{15}H_{30}O$	40.61	1697	1694	0.102 ± 0.011	0.325 ± 0.047	0.34 ± 0.12	0.224±0.045

Table 3.4 continues

Methyl tetradecanoate	$C_{15}H_{30}O_2$	41.58	1722	1721		0.06 ± 0.035	0.097 ± 0.037	0.276±0.007
Tetradecanoic acid (Myristric acid)	$C_{14}H_{28}O_2$	42.89	1765	1758	0.069 ± 0.014	0.208 ± 0.011	0.095 ± 0.011	0.091±0.024
Ethyl myristate	$C_{16}H_{32}O_2$	44.02	1793	1790	0.089 ± 0.021	1.261 ± 0.132	0.231 ± 0.051	0.286±0.037
6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	45.68	1844	1841	0.468 ± 0.08	0.454 ± 0.042	0.106 ± 0.015	0.103±0.009
Benzyl salicylate	$C_{13}H_{18}O$	46.41	1868	1864		0.06 ± 0.035	0.097 ± 0.037	0.276±0.007
(5E.9E)-Farnesyl acetone	$C_8H_8O_3$	47.85	1913	1914	2.428 ± 0.582	0.467 ± 0.058	2.174 ± 0.245	0.354±0.035
Methyl hexadecanoate (Methyl palmitate)	$C_{12}H_{24}O_2$	48.05	1921	1924		0.079 ± 0.012	0.133 ± 0.02	0.194±0.014
cis-9-Hexadecenoic acid (Palmitoleic acid)	$C_{14}H_{30}$	48.69	1953	1955	0.152 ± 0.013	0.252 ± 0.027	0.328 ± 0.017	1.818±0.147
Hexadecanoic acid (Palmitic acid)	$C_{12}H_{24}O$	48.77	1959	1959				
Ethyl 9-hexadecenoate	$C_{13}H_{20}O$	49.03	1977	1971	0.308 ± 0.042	0.221 ± 0.05	0.087 ± 0.014	0.035±0.005
Ethyl hexadecanoate (Ethyl palmitate)	$C_{13}H_{22}O$	49.41	1992	1991		0.512 ± 0.025		
n-Eicosane	$C_{13}H_{22}O$	49.5	2000	1995	0.212 ± 0.036	10.27 ± 0.48	0.668 ± 0.042	1.850±0.151
Isopropyl hexadecanoate	$C_{12}H_{26}O$	49.94	2024	2022	0.069 ± 0.01		0.235 ± 0.04	
Methyl linoleate	$C_{13}H_{18}O$	51.01	2095	2091	0.062 ± 0.008	0.175 ± 0.039	0.081 ± 0.019	
Methyl oleate	$C_{13}H_{16}O$	51.03	2103	2092		0.129 ± 0.01	0.151 ± 0.017	0.706±0.081
9-Octadecenoic acid (Oleic acid)	$C_{13}H_{26}O$	51.26	2110	2109		0.076 ± 0.015		0.451±0.045
(Z,Z)-9,12-octadecadienoic acid (Linoleic acid)	$C_{15}H_{32}$	51.56	2132	2134				
Ethyllinoleate	$C_{15}H_{24}$	51.83	2159	2156	0.092 ± 0.032	0.39 ± 0.028	0.128 ± 0.01	0.064±0.007
Octadecanoic acid (Stearic acid)	$C_{11}H_{16}O_2$	52.06	2172	2175		1.228 ± 0.033		0.157±0.016
Ethyl stearate	$C_{12}H_{24}O_2$	52.22	2190	2188				
Octadecyl Octanoate	$C_{12}H_{14}O_4$	57.5	2765	2766	0.083 ± 0.02	0.147 ± 0.011	0.309 ± 0.095	0.081±0.006

Table 3.5 Identified volatile compounds with PA fiber in goji berry samples

	Area. % by GC/MS (x±s)							
Compounds	**Formula**	**t_R (min)**	**RI_{lit}**	**RI_{calc}**	**Black GB**	**Hybrid GB**	**Natural GB**	**GB from market**
Heptane	C_7H_{16}	3.14	700	702	0.194 ± 0.045	0.14 ± 0.013	0.202 ± 0.044	0.148 ± 0.021
Isoamylol	$C_5H_{12}O$	3.66	731	732	0.754 ± 0.015	0.146 ± 0.025	0.265 ± 0.037	
1-Pentanol	$C_5H_{12}O$	4.23	762	765	0.388 ± 0.058		0.425 ± 0.029	0.295 ± 0.034
2.6-dimethyl-3-Heptene	C_9H_{18}	5.21	815	816	0.849 ± 0.081	0.186 ± 0.014	0.464 ± 0.017	0.298 ± 0.05
Furfural	$C_5H_4O_2$	5.59	828	832			0.336 ± 0.034	
Methyl tiglate	$C_6H_{10}O_2$	6.4	863	764	0.235 ± 0.047	0.416 ± 0.037	0.105 ± 0.021	
Styrene	C_8H_8	6.75	880	879		0.484 ± 0.045	0.611 ± 0.025	0.099 ± 0.032
(Z)-4-Heptenal	$C_7H_{12}O$	7.1	893	893			0.291 ± 0.028	0.31 ± 0.013
Heptanal	$C_7H_{14}O$	7.32	901	901	0.255 ± 0.028		0.217 ± 0.069	0.21 ± 0.045
Butyrolactone	$C_4H_6O_2$	7.6	908	908	1.654 ± 0.112	0.235 ± 0.02	0.975 ± 0.021	0.988 ± 0.064
2.5-Hexanedione	$C_6H_{10}O_2$	8.02	920	919	0.419 ± 0.131	0.12 ± 0.021	0.59 ± 0.079	0.361 ± 0.036
2-propenyl 3-Methylbutanoate	$C_8H_{14}O_2$	8.89	931	941	0.718 ± 0.045	0.15 ± 0.012	0.561 ± 0.047	0.447 ± 0.042
(2E)-Heptenal	$C_7H_{12}O$	9.11	947	949	0.191 ± 0.024	0.356 ± 0.032	0.348 ± 0.06	0.343 ± 0.031
Benzaldehyde	C_7H_6O	9.35	952	953	1.078 ± 0.04		0.95 ± 0.023	1.059 ± 0.108
Hexanoic acid	$C_6H_{12}O_2$	9.89	967	966				
1-Octen-3-one	$C_8H_{14}O$	10.14	972	973	0.104 ± 0.023		0.095 ± 0.026	
1-Octen-3-o1	$C_8H_{16}O$	10.18	974	974	0.43 ± 0.011	0.629 ± 0.134	0.319 ± 0.031	0.229 ± 0.029
2-Pentylfuran	$C_9H_{14}O$	10.71	984	987			0.173 ± 0.017	
Benzyl alcohol	C_7H_8O	12.47	1026	1028	0.894 ± 0.039	0.68 ± 0.02	0.498 ± 0.065	0.107 ± 0.016
Benzeneacetaldehyde	C_8H_8O	12.86	1036	1036	1.119 ± 0.248	0.242 ± 0.052	0.663 ± 0.047	0.659 ± 0.03
(E)-2-Octen-1-al	$C_8H_{14}O$	13.41	1049	1049	0.264 ± 0.032	0.128 ± 0.024	0.398 ± 0.034	0.357 ± 0.035
2-Acetylpyrrole	C_6H_7NO	13.69	1054	1055	2.414 ± 0.255		0.45 ± 0.036	3.936 ± 0.386
1-Octanol	$C_8H_{18}O$	14.29	1063	1067	0.191 ± 0.024		0.173 ± 0.081	0.17 ± 0.024

Table 3.5 continues

tetramethyl-Pyrazine	$C_8H_{12}N_2$	14.84	1083	1080		0.038 ± 0.009		0.164 ± 0.047
2-Nonanone	$C_9H_{18}O$	15.2	1087	1088		0.181 ± 0.018		
Nonanal	$C_9H_{18}O$	15.77	1100	1100	1.107 ± 0.168	0.515 ± 0.023	0.679 ± 0.07	0.518 ± 0.087
Benzeneethanol	$C_8H_{10}O$	16.06	1107	1107	1.199 ± 0.31	1.552 ± 0.171	0.705 ± 0.189	0.499 ± 0.025
Methyl octanoate	$C_9H_{18}O_2$	16.8	1123	112	0.292 ± 0.045		0.321 ± 0.034	0.396 ± 0.019
2.3-dihydro-3.5-dihydroxy-6-methyl-4H-Pyran-4-one	$C_6H_8O_4$	17.37	1134	1135	1.63 ± 0.178	0.212 ± 0.032	0.65 ± 0.087	3.59 ± 0.434
Menthone	$C_{10}H_{18}O$	18.03	1148	1149	0.12 ± 0.01	0.195 ± 0.029		
(E)-2-Nonenal	$C_9H_{16}O$	18.29	1157	1155	0.205 ± 0.075		0.098 ± 0.014	
1-Nonanol	$C_9H_{20}O$	18.94	1169	1169	0.443 ± 0.065	0.615 ± 0.049	0.318 ± 0.031	0.286 ± 0.018
Naphthalene	$C_{10}H_8$	19.15	1178	1175	0.25 ± 0.098		0.142 ± 0.053	
Benzoic acid	$C_7H_6O_2$	19.36	1178	1178	0.185 ± 0.027			
Methyl salicylate	$C_8H_8O_3$	19.88	1190	1189	0.515 ± 0.075	0.589 ± 0.059	0.272 ± 0.073	
Safranal	$C_{10}H_{14}O$	20.05	1197	1193	0.145 ± 0.024	0.265 ± 0.025	0.221 ± 0.062	0.225 ± 0.047
Dodecane	$C_{12}H_{26}$	20.22	1200	1197	0.272 ± 0.01	0.214 ± 0.074	0.151 ± 0.073	
Decanal	$C_{10}H_{20}O$	20.43	1201	1201	0.33 ± 0.024	0.157 ± 0.043	0.671 ± 0.12	0.268 ± 0.054
β-Cyclocitral	C_8H_8O	21.05	1217	1215	1.097 ± 0.281	0.296 ± 0.094	2.04 ± 0.56	1.585 ± 0.158
5-Hydroxymethylfurfural	$C_6H_6O_3$	21.41	1224	1223	1.34 ± 0.0358		0.52 ± 0.099	0.707 ± 0.087
Methylethylmaleimide	$C_7H_9NO_2$	21.61	1235	1227	0.235 ± 0.039		0.301 ± 0.034	0.182 ± 0.034
2-Phenylethyl acetate	$C_{10}H_{12}O_2$	22.67	1254	1251		0.159 ± 0.027		
Nonanoic acid	C9H18O2	23.5	1267	1269	0.153 ± 0.027	0.407 ± 0.094	0.234 ± 0.024	0.763 ± 0.068
2-Undecanone	$C_{11}H_{22}O$	24.39	1293	1289			0.759 ± 0.039	0.458 ± 0.087
Tridecane	$C_{13}H_{28}$	24.7	1300	1296				
2-Undecanol	$C_{11}H_{24}O$	24.93	1303	1301	0.134 ± 0.036		0.125 ± 0.05	
4-Vinylguaiacol	$C_9H_{10}O_2$	25.19	1309	1307	0.354 ± 0.07	0.57 ± 0.008	0.824 ± 0.068	0.16 ± 0.024
Limonene aldehyde	$C_{11}H_{18}O$	26.02	1328	1327		0.169 ± 0.045	0.136 ± 0.05	0.349 ± 0.063
Eugenol	$C_{10}H_{12}O_2$	27.09	1356	1352			0.244 ± 0.024	0.196 ± 0.047

Table 3.5 continues

Nonan-1.4-olide	$C_9H_{16}O_2$	27.26	1358	1356	0.322 ± 0.072	0.255 ± 0.014	0.313 ± 0.025	0.149 ± 0.024
Decanoic acid	$C_{10}H_{20}O_2$	27.77	1364	1368	0.41 ± 0.034		1.361 ± 0.147	1.159 ± 0.104
Table 3.5 continued	Formula	tR (min)	RIlit	RIcalc	Black GB	Hybrid GB	Natural GB	GB from market
β-(E)-Damascenone	C13H18O	28.26	1383	1379	0.186 ± 0.064		0.237 ± 0.015	0.143 ± 0.012
Vanillin	C8H8O3	28.78	1393	1391			0.082 ± 0.01	
Decanoic acid. ethyl ester	C12H24O2	28.84	1392	1392	0.67 ± 0.065	0.414 ± 0.057	0.376 ± 0.057	0.116 ± 0.014
Tetradecane	C14H30	29.05	1400	1397			0.162 ± 0.077	6.176 ± 0.236
Dodecanal	C12H24O	29.3	1408	1404	0.187 ± 0.018	0.174 ± 0.034	0.256 ± 0.035	0.13 ± 0.032
α-Ionone	C13H20O	30.09	1426	1423	0.295 ± 0.065	0.167 ± 0.027	0.325 ± 0.072	0.179 ± 0.041
Dihydro-β-ionone	C13H22O	30.57	1434	1434				0.197 ± 0.035
Geranylacetone	C13H22O	31.15	1453	1448	0.376 ± 0.052	0.36 ± 0.023	1.304 ± 0.058	0.801 ± 0.144
1-Dodecanol	C12H26 O	32	1469	1469		0.151 ± 0.023	0.151 ± 0.012	0.293 ± 0.056
3.4-Dehydro-β-ionone	C13H18O	32.39	1485	1479				0.14 ± 0.024
5-Methyl-2-phenyl-2-hexenal	C13H16O	32.63	1485	1485				0.212 ± 0.021
2-Tridecanone	C13H26O	32.9	1495	1491		0.183 ± 0.029	0.547 ± 0.033	0.283 ± 0.049
Pentadecane	C15H32	33.07	1500	1496	0.316 ± 0.047		0.184 ± 0.012	0.27 ± 0.032
Valencene	C15H24	33.21	1496	1499	0.249 ± 0.057	0.231 ± 0.032	0.346 ± 0.013	0.354 ± 0.064
Dihydroactinidiolide	C11H16O2	34.02	1525	1521	0.333 ± 0.064	0.312 ± 0.018	0.692 ± 0.14	1.079 ± 0.245
Dodecanoic acid	C12H24O2	35.7	1565	1563	0.428 ± 0.043	0.164 ± 0.027	0.361 ± 0.031	0.173 ± 0.063
Diethyl phthalate (plastizer. comtaminant)	C12H14O4	36.69	1590	1588	0.342 ± 0.034	0.315 ± 0.005	0.242 ± 0.072	0.684 ± 0.042
Ethyl laurate	C14H28O2	36.76	1594	1591		0.129 ± 0.029	0.154 ± 0.014	0.484 ± 0.052
Tetradecanal	C14H28O	37.41	1611	1607	0.181 ± 0.047	0.132 ± 0.016	0.181 ± 0.017	0.19 ± 0.025
Benzophenone	C13H10O	37.81	1621	1618	1.864 ± 0.365	1.001 ± 0.147	1.98 ± 0.285	1.413 ± 0.345
(1-butylheptyl)-Benzene	C17H28	38.27	1633	1631				1.697 ± 0.374
3-Keto-β-ionone	C13H18O2	39.48	1661	1663				0.2 ± 0.034
1-Tetradecanol	C14H30O	39.78	1671	1672	0.659 ± 0.067	0.456 ± 0.02	0.911 ± 0.048	0.493 ± 0.074

Table 3.5 continues

2-Pentadecanone	C15H30O	40.61	1697	1694	0.24 ± 0.034	0.219 ± 0.051	0.385 ± 0.036	0.408 ± 0.024
Methyl tetradecanoate	C15H30O2	41.58	1722	1721				0.389 ± 0.014
Tetradecanoic acid (Myristric acid)	C14H28O2	42.89	1765	1758	0.344 ± 0.074	0.305 ± 0.035	0.464 ± 0.012	0.586 ± 0.026
Ethyl myristate	C16H32O2	44.02	1793	1790	0.25 ± 0.026	0.202 ± 0.041	0.186 ± 0.034	0.189 ± 0.018
6.10.14-trimethyl-2-Pentadecanone	C18H36O	45.68	1844	1841	0.195 ± 0.029	0.125 ± 0.012		0.188 ± 0.032
Table 3.5 continued	Formula	tR (min)	RIlit	RIcalc	Black GB	Hybrid GB	Natural GB	GB from market
Benzyl salicylate	C16H22O4	46.41	1868	1864	0.834 ± 0.153	0.388 ± 0.014	1.015 ± 0.09	0.488 ± 0.025
(5E.9E)-Farnesyl acetone	C18H30O	47.85	1913	1914	0.151 ± 0.054	0.126 ± 0.012	0.299 ± 0.013	0.829 ± 0.047
Methyl hexadecanoate (Methyl palmitate)	C17H34O2	48.05	1921	1924	0.157 ± 0.034	0.11 ± 0.014	0.184 ± 0.042	1.411 ± 0.6
cis-9-Hexadecenoic acid (Palmitoleic acid)	C16H30O2	48.69	1953	1955	0.109 ± 0.014		0.154 ± 0.024	
Hexadecanoic acid (Palmitic acid)	C16H32O2	48.77	1959	1959	1.242 ± 0.341	2.799 ± 0.778	2.34 ± 0.248	0.961 ± 0.065
Ethyl 9-hexadecenoate	C18H34O2	49.03	1977	1972	0.548 ± 0.054	0.506 ± 0.069	1.099 ± 0.237	0.138 ± 0.024
Ethyl hexadecanoate (Ethyl palmitate)	C18H36O2	49.41	1992	1991	0.988 ± 0.263	30.719 ± 4.104	0.688 ± 0.046	1.855 ± 0.235
n-Eicosane	C20 H42	49.5	2000	1995	0.31 ± 0.058		0.101 ± 0.018	2.094 ± 0.542
Isopropyl hexadecanoate	C19H38O2	49.94	2024	2022	0.243 ± 0.051	0.167 ± 0.034	0.27 ± 0.019	0.286 ± 0.044
Methyl linoleate	C19H34O2	51.01	2095	2091	2.033 ± 0.15	0.708 ± 0.045	1.451 ± 0.189	3.821 ± 0.089
Methyl oleate	C19H36O2	51.03	2103	2092	0.532 ± 0.15	0.301 ± 0.028	1.028 ± 0.166	1.586 ± 0.166
9-Octadecenoic acid (Oleic acid)	C18H34O2	51.26	2110	2109	0.265 ± 0.02		0.087 ± 0.025	0.798 ± 0.087
(Z.Z)-9.12-octadecadienoic acid (Linoleic acid)	C18H32O2	51.56	2132	2134	0.445 ± 0.078	0.472 ± 0.015	0.69 ± 0.018	0.543 ± 0.045
Ethyllinoleate	C18H36O2	51.83	2159	2156	0.191 ± 0.042	15.538 ± 1.54	0.485 ± 0.038	0.672 ± 0.065
Octadecanoic acid (Stearic acid)	C18H36O2	52.06	2172	2175	0.488 ± 0.081	0.432 ± 0.047	0.364 ± 0.039	0.588 ± 0.076
Ethyl stearate	C20H40O2	52.22	2190	2188	0.391 ± 0.87	0.981 ± 0.031	0.669 ± 0.062	0.763 ± 0.048
Octadecyl Octanoate	C26H52O2	57.5	2765	2766		0.201 ± 0.038	0.274 ± 0.039	0.312 ± 0.047

Table 3.6 Identified volatile compounds with HD method in goji berry samples

	Area. % by GC/MS (x±s)							
Compounds	**Formula**	**t_R (min)**	**RI_{lit}**	**RI_{calc}**	**Black GB**	**Hybrid GB**	**Natural GB**	**GB from market**
Heptane	C_7H_{16}	3.14	700	702			1.042	
Isoamylol	$C_5H_{12}O$	3.66	731	732	0.046		0.058	
1-Pentanol	$C_5H_{12}O$	4.23	762	765	0.056	0.073	0.051	
2.6-dimethyl-3-Heptene	C_9H_{18}	5.21	815	816			0.041	
Furfural	$C_5H_4O_2$	5.59	828	832			0.016	
Methyl tiglate	$C_6H_{10}O_2$	6.4	863	764			0.034	
Styrene	C_8H_8	6.75	880	879	0.034		0.033	
(Z)-4-Heptenal	$C_7H_{12}O$	7.1	893	893			0.100	
Heptanal	$C_7H_{14}O$	7.32	901	901			0.044	
Butyrolactone	$C_4H_6O_2$	7.6	908	908	0.034		3.750	
2.5-Hexanedione	$C_6H_{10}O_2$	8.02	920	919			0.018	
2-propenyl 3-Methylbutanoate	$C_8H_{14}O_2$	8.89	931	941			0.060	
(2E)-Heptenal	$C_7H_{12}O$	9.11	947	949	0.903		0.029	
Benzaldehyde	C_7H_6O	9.35	952	953				0.047
Hexanoic acid	$C_6H_{12}O_2$	9.89	967	966				
1-Octen-3-one	$C_8H_{14}O$	10.14	972	973	0.025		0.014	
1-Octen-3-o1	$C_8H_{16}O$	10.18	974	974				
2-Pentylfuran	$C_9H_{14}O$	10.71	984	987	0.074	0.043	0.016	
Benzyl alcohol	C_7H_8O	12.47	1026	1028	0.053	0.013	0.045	
Benzeneacetaldehyde	C_8H_8O	12.86	1036	1036			0.038	
(E)-2-Octen-1-al	$C_8H_{14}O$	13.41	1049	1049			0.060	
2-Acetylpyrrole	C_6H_7NO	13.69	1054	1055	0.017		0.049	
1-Octanol	$C_8H_{18}O$	14.29	1063	1067				

Table 3.6 continues

tetramethyl-Pyrazine	$C_8H_{12}N_2$	14.84	1083	1080	0.073		0.059	
2-Nonanone	$C_9H_{18}O$	15.2	1087	1088			0.026	
Nonanal	$C_9H_{18}O$	15.77	1100	1100				
Benzeneethanol	$C_8H_{10}O$	16.06	1107	1107		0.022		
Methyl octanoate	$C_9H_{18}O_2$	16.8	1123	112				
2.3-dihydro-3.5-dihydroxy-6-methyl-4H-Pyran-4-one	$C_6H_8O_4$	17.37	1134	1135			0.031	
Menthone	$C_{10}H_{18}O$	18.03	1148	1149				
(E)-2-Nonenal	$C_9H_{16}O$	18.29	1157	1155			0.021	
1-Nonanol	$C_9H_{20}O$	18.94	1169	1169	0.034			
Naphthalene	$C_{10}H_8$	19.15	1178	1175				
Benzoic acid	$C_7H_6O_2$	19.36	1178	1178				
Methyl salicylate	$C_8H_8O_3$	19.88	1190	1189			0.044	
Safranal	$C_{10}H_{14}O$	20.05	1197	1193			0.018	
Dodecane	$C_{12}H_{26}$	20.22	1200	1197	0.049		0.026	
Decanal	$C_{10}H_{20}O$	20.43	1201	1201				
β-Cyclocitral	C_8H_8O	21.05	1217	1215			0.018	
5-Hydroxymethylfurfural	$C_6H_6O_3$	21.41	1224	1223			0.026	
Methylethylmaleimide	$C_7H_9NO_2$	21.61	1235	1227	0.045			
2-Phenylethyl acetate	$C_{10}H_{12}O_2$	22.67	1254	1251	0.176		0.012	
Nonanoic acid	C9H18O2	23.5	1267	1269	0.314		0.100	
2-Undecanone	$C_{11}H_{22}O$	24.39	1293	1289				
Tridecane	$C_{13}H_{28}$	24.7	1300	1296	0.038			
2-Undecanol	$C_{11}H_{24}O$	24.93	1303	1301			0.015	
4-Vinylguaiacol	$C_9H_{10}O_2$	25.19	1309	1307	0.044	0.139		
Limonene aldehyde	$C_{11}H_{18}O$	26.02	1328	1327	0.018	0.016	0.032	
Eugenol	$C_{10}H_{12}O_2$	27.09	1356	1352	0.091	0.288	0.017	0.015

Table 3.6 continues

Nonan-1.4-olide	$C_9H_{16}O_2$	27.26	1358	1356	0.315	0.020	0.034	
Decanoic acid	$C_{10}H_{20}O_2$	27.77	1364	1368		0.562	0.038	0.093
β-(E)-Damascenone	C13H18O	28.26	1383	1379				
Vanillin	C8H8O3	28.78	1393	1391	0.809	0.148		0.019
Decanoic acid. ethyl ester	C12H24O2	28.84	1392	1392				
Tetradecane	C14H30	29.05	1400	1397				
Dodecanal	C12H24O	29.3	1408	1404		0.154	0.060	
α-Ionone	C13H20O	30.09	1426	1423	0.029	0.013		
Dihydro-β-ionone	C13H22O	30.57	1434	1434				
Geranylacetone	C13H22O	31.15	1453	1448	0.015	0.134		
1-Dodecanol	C12H26 O	32	1469	1469				
3.4-Dehydro-β-ionone	C13H18O	32.39	1485	1479	0.045	0.012		
5-Methyl-2-phenyl-2-hexenal	C13H16O	32.63	1485	1485	0.014	0.028	0.025	0.036
2-Tridecanone	C13H26O	32.9	1495	1491	0.909	0.125	0.014	0.020
Pentadecane	C15H32	33.07	1500	1496			0.039	
Valencene	C15H24	33.21	1496	1499	0.131	0.133	0.105	0.084
Dihydroactinidiolide	C11H16O2	34.02	1525	1521	0.050	0.045	0.028	0.136
Dodecanoic acid	C12H24O2	35.7	1565	1563	1.325	1.661	0.025	1.049
Diethyl phthalate (plastizer. comtaminant)	C12H14O4	36.69	1590	1588	0.112	0.188	0.250	0.174
Ethyl laurate	C14H28O2	36.76	1594	1591	0.140	0.023	0.000	0.000
Tetradecanal	C14H28O	37.41	1611	1607	0.074	0.029	0.027	0.017
Benzophenone	C13H10O	37.81	1621	1618	0.236	0.099	0.017	0.030
(1-butylheptyl)-Benzene	C17H28	38.27	1633	1631	0.081	0.042	0.019	
3-Keto-β-ionone	C13H18O2	39.48	1661	1663	0.843	1.249	0.039	0.112
1-Tetradecanol	C14H30O	39.78	1671	1672	6.135	3.737	1.059	1.228
2-Pentadecanone	C15H30O	40.61	1697	1694	0.174	0.271	0.188	0.100

Table 3.6 continues

Methyl tetradecanoate	C15H30O2	41.58	1722	1721	0.084	0.135	0.098	0.103
Tetradecanoic acid (Myristric acid)	C14H28O2	42.89	1765	1758	2.985	2.421	0.340	1.148
Ethyl myristate	C16H32O2	44.02	1793	1790	0.609	1.159	1.410	0.356
6.10.14-trimethyl-2-Pentadecanone	C18H36O	45.68	1844	1841	0.460	0.348	0.160	0.151
Table 3.6 continued	Formula	tR (min)	RIlit	RIcalc	Black GB	Hybrid GB	Natural GB	GB from market
Benzyl salicylate	C16H22O4	46.41	1868	1864	3.945	2.188	2.687	1.184
(5E.9E)-Farnesyl acetone	C18H30O	47.85	1913	1914	0.636	1.564	0.209	0.512
Methyl hexadecanoate (Methyl palmitate)	C17H34O2	48.05	1921	1924	0.092	0.344	1.052	20.108
cis-9-Hexadecenoic acid (Palmitoleic acid)	C16H30O2	48.69	1953	1955	0.056	0.033	0.150	0.028
Hexadecanoic acid (Palmitic acid)	C16H32O2	48.77	1959	1959	1.296	0.466	9.045	9.887
Ethyl 9-hexadecenoate	C18H34O2	49.03	1977	1971	11.173	0.101	0.153	0.257
Ethyl hexadecanoate (Ethyl palmitate)	C18H36O2	49.41	1992	1991	1.281	16.073	11.143	9.887
n-Eicosane	C20 H42	49.5	2000	1995	0.216	0.202	4.168	1.050
Isopropyl hexadecanoate	C19H38O2	49.94	2024	2022	0.227	0.900	0.201	0.289
Methyl linoleate	C19H34O2	51.01	2095	2091	0.729	0.349	0.919	4.266
Methyl oleate	C19H36O2	51.03	2103	2092	0.605	0.450	0.885	2.991
9-Octadecenoic acid (Oleic acid)	C18H34O2	51.26	2110	2109	0.191	0.080	0.296	0.697
(Z.Z)-9.12-octadecadienoic acid (Linoleic acid)	C18H32O2	51.56	2132	2134	8.296	8.965	3.983	5.709
Ethyllinoleate	C18H36O2	51.83	2159	2156	3.358	10.380	0.506	5.246
Octadecanoic acid (Stearic acid)	C18H36O2	52.06	2172	2175	0.286	0.021	0.866	0.025
Ethyl stearate	C20H40O2	52.22	2190	2188	0.594	1.645	1.313	0.964
Octadecyl Octanoate	C26H52O2	57.5	2765	2766	0.058		0.232	

CHAPTER FOUR

CONCLUSIONS

In this study. four different goji berry samples gotten from Turkey in September 2020 were evaluated and compared with one another. The result showed that the GB from the market had the highest EC_{50} value. Among the samples. Black GB showed better result against the DPPH radical scavenging (had the lowest EC_{50} value). that is. it had the highest antioxidant activity. And when compared with other literatures. it showed similar trend in which the GB types with the lowest EC_{50} values had the highest antioxidant activities.

Each of the goji berry samples underwent acid digestion in three parallels and their elemental compositions were analyzed using ICP-OES. The values obtained for the macro - (Calcium. Sodium. Magnesium. Phosphorus. and Potassium) and microelements (Selenium. Zinc. Manganese. Copper. Iron) and some trace elements conferred the quality of mineral supplementation on these samples.

The phenolic profile of the four dried goji berries were investigated using HPLC. Twelve phenolic compounds were identified in the goji berry samples. Hydroxycinnamic acids. followed by hydroxybenzoic acids. and flavonoids were the most abundant compounds. However. among the 12 compounds. rutin has the largest mass fraction as shown in another study by Carvalho et al. (2016). In addition. the phenolic compounds found in this study were identified in other literatures (De Moura et al.. 2018; Donno et al.. 2015; Carvalho et al., 2016).

Lastly. the volatile compounds of the four dried goji berries were evaluated using GC-MS by means of the HS-SPME method with commercial fibers (DVB/CAR/PDMS and PA). and HD method. A total of 96 volatile compounds were identified out of the 188 detected. which include 17 aldehydes. 12 alcohols. 21 esters. 18 ketones. 1 terpene. 2 furans. 10 fatty acids. 1 aromatic carboxylic acid. 1 aromatic compound. 6 alkanes. 1 alkene. 1 pyrazine. 1 pyrrole. 1 naphthalene. and 3 miscellanous compounds. It was noted that some of the volatile compounds such as (E)-2-hexenal. nonanal. 1-octen-3-ol. linalool. etc which were important volatiles in

Ningxia goji berries (Lu et al.. 2009) were also found in this study. Also. the major fatty acids found by Zhao. (2018) which include linoleic acid. oleic acid. palmitic acid. and stearic acid were identified in this study as well. Furthermore. the similar components found in the oil of *L. barbarum* by Altintas. (2006) which include ethyl hexadecanoate. myristic acid and hexadecenoic acid and the essential oil in *L. ruthenicum* was ethyl hexadecanoate (5.8%). and ethyl linoleate were revealed in this work. too. Lastly. it was concluded that more volatile compounds were identified using DVB/CAR/PDMS and PA SPME fibers compared to HD method.

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