DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

CHROMATOGRAFIC ANALYSIS OF PHENOLIC COMPOSITIONS OF SOME FOODS AFTER ENRICHMENT BY MICROEXTRACTION TECHNIQUES

by

Paniz TASHAKKORI

February, 2022

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Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled "**CHROMATOGRAFIC ANALYSIS OF PHENOLIC COMPOSITIONS OF SOME FOODS AFTER ENRICHMENT BY MICROEXTRACTION TECHNIQUES"** 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 Doctor of Philosophy.

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ABSTRACT

In this study, solid-phase micro-extraction methods were developed using new coated fibers including graphene oxide and clay modified with ionic liquids for the separation and extraction of phenolic compounds, and then their determinations were performed by gas chromatography-mass spectrometry and liquid chromatography methods. Ionic liquids with methyl- and benzylimidazolium cation and bromide, tetrafluoroborate and bis (trifluoromethane) sulfonimide anions were synthesized, fibers containing graphene oxide-ionic liquid and clay-ionic liquid were prepared and all of them were characterized. Phenolic compounds were derivatized using trimethylsilane reagents before determination by gas chromatography. The optimum conditions like temperature, time and reagent volume were optimized for derivatization. In the immersion solid phase microextraction-gas chromatography method, pH, extraction temperature, extraction time, salt effect, stirring speed, desorption temperature and desorption time were studied. Analytical parameters (linear range, detection limit, repeatability, and reproducibility) were determined for phenolic compounds. Elution solvent type, elution temperature and elution time were optimized in liquid chromatography method. The extraction abilities of the prepared fibers were found higher than the commercial fibers. The methods developed have been successfully applied to a several of wine or fruit juice samples to show the accuracy of the developed methods by standard spiking of standard of phenolic compounds and high recovery was obtained.

Keywords: Phenolic compounds, solid phase microextraction, GC-MS, LC/DAD.

MİKROEKSTRAKSİYON TEKNİKLERİ İLE ZENGİNLEŞTİRME SONRASI BAZI GIDALARIN FENOLİK BİLEŞİMLERİNİN KROMATOGRAFİK ANALİZİ

ÖZ

Bu çalışmada, fenolik bileşiklerin ekstraksiyonu için yeni iyonik sıvı ile modifiye edilmiş grafen oksit ve iyonik sıvı ile modifiye edilmiş kil kaplı fiberler kullanılarak katı faz mikroekstraksiyonu yöntemleri geliştirildi ve sonrasında gaz kromatografisi-kütle spektrometrisi ve sıvı kromatografisi yöntemi ile tayinleri gerçekleştirildi. Metil- ve benzilimidazolyum katyonlu bromür, tetrafloroborat ve bis (triflorometan) sülfonimid anyonlu iyonik sıvılar sentezlendi, grafen oksit-iyonik sıvı ve kil-iyonik sıvı içeren fiberler hazırlandı ve karakterize edildiler. Gaz kromatografisi ile tayin öncesi fenolik bileşikler trimetilsilan reaktifleri kullanılarak türevlendirildi. Türevlendirme için sıcaklık, süre ve reaktif hacmi optimize edildi. Daldırmalı katı faz mikroekstraksiyonu yönteminde pH, ekstraksiyon sıcaklığı, ekstraksiyon süresi, tuz etkisi, karıştırma hızı, desorpsiyon sıcaklığı ve desorpsiyon süresi optimize edildi. Optimum koşullar altında, fenolik bileşikler için analitik parametreler (çalışma aralığı, gözlenebilme sınırı, tekrarlanabilirlik) belirlendi. Sıvı kromatografisi yöntemi ile tayinde elüsyon çözücüsü türü, elüsyon sıcaklığı ve elüsyon süresi optimize edildi. Hazırlanan fiberlerin ekstraksiyon kabiliyeti ticari fiberlerden daha yüksek elde edildi. Geliştirilen yöntemler, çeşitli şarap veya meyve suyu örneklerine başarıyla uygulandı. Geliştirilen yöntemlerin doğruluğunu göstermek için gerçek örneklere standart fenolik bileşik eklemesi yapıldı ve yüksek geri kazanım elde edildi.

Anahtar kelimeler: Fenolik bileşikler, katı faz mikroekstraksiyonu, GC-MS, LC/DAD

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CHAPTER ONE
INTRODUCTION

1.1 Phenolic Compounds

Organic compounds containing at least one aromatic ring and one or more hydroxyl group in this ring are called phenolic compounds or polyphenols. It is known that the simplest phenolic substance is benzene, that is, phenol, which contains one hydroxyl group, and other phenolic substances are derived from it. Phenolic compounds are secondary metabolites found in plants in large amounts. These compounds take part highly in the human diet. Fruits, vegetables, and beverages (wine, beer, tea, etc.) are the main source of phenolic compounds in the human diet. Herbal products have antioxidant properties because of their phenolic compounds content. These compounds prevent the formation of many diseases such as cancer, diabetes, Alzheimer's and heart diseases by obstructing the free radical formation (Jiang et al., 2006). In the literature, there are many studies that reveal the pro-oxidant properties of plant-derived phenolic compounds as well as their antioxidant properties (Letizia et al., 2015). So, high consumption of plant-based foods is recommended in the human diet such as 25 mg of fruit, vegetables, coffee, tea per g per day (Tianet et al., 2017). There has been much interest in research that describes the potential healthy nutritional impact and metabolism of phenolic compounds and their qualitative and quantitative analysis in herbal products by different analytical techniques.

Polyphenolic compounds are generally categorized into four groups; flavonoids, phenolic acids, polyphenolic amides, and other polyphenols. The flavonoid group includes 60% of all polyphenolic compounds such as catechins, anthocyanins, kaempferol, and quercetin that take place in some vegetables and fruits (red cabbage, onions, apples, etc.). Phenolic acids make up approximately 30% of polyphenolic compounds. Avenanthramides in oats and capsaicinoids in chili peppers are known as polyphenolic amides. Apart from, lignans in sesame seeds and whole grains, ellagic acid in berries and resveratrol in red wine are classified as other polyphenolic compounds (Tsao, 2010). The farming way, transportation, storing, producing

conditions of herbal-based foods and also their origin and ripeness are effective in types and amounts of phenolic compounds found in that foods. Discussions about the majority and important ones of polyphenolic compounds in plants or fruits and their biological effects on human health may help create novel directions for future investigations.

1.1.1 Flavonoids

Flavonoids are commonly found in plants to perform many functions. Flavonoids are the most important plant pigments for flowering and produce yellow or red-blue pigments in leaves designed to attract pollinators. In more advanced plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation, and flower pigmentation. They can also function as a chemical stimulant, physiological regulator, and cell cycle inhibitor (Guo et al., 2019). Flavonoids have six main groups which are flavonoids, including flavones, anthocyanidins, flavanones, isoflavones, flavonols and flavanols. Flavonoids take attention with their antioxidant, antimutagenic, antiproliferative, antitumor, antiviral, and anti-inflammatory properties. Epidemiological studies have shown the importance of flavonoid compounds in reducing the risk of cardiovascular diseases and cancer (Fan et al., 2019). So, the most common type of polyphenolic compounds, flavonoids are found in the human diet.

1.1.1.1 Flavones

This group includes luteolin and apigenin are known for their antioxidant properties and delay the metabolism of drugs. Flavones are mostly present in many kinds of herbs such as parsley, celery, etc.

1.1.1.2 Anthocyanidins

Anthocyanidins (malvidin, pelargonidin, peonidin and cyaniding) are effective in heart health as an antioxidant and help prevent obesity, diabetes. They are found in red colour-based fruits like plums, blue berry, grapes, pomegranate, etc.

1.1.1.3 Flavonones

Flavanones are mostly present in orange, grapefruit, tangerine, and other similar fruits. They have antioxidant and anti-inflammatory activity due to including hesperetin, eriodictyol, and naringenin, etc. These compounds are important in cardiovascular health.

1.1.1.4 Isoflavones

These natural chemical compounds are known as phytoestrogens which behave as estrogen hormones. They can be useful to decrease the cancer possibility based on hormonal ones like prostate, breast, and endometrial. The effect of flavones including genistein, glycitein, and daidzein on cancer is not clear because they can behave as antioxidants or oxidants. This group is found at high concentrations in legumes and is also found in soybeans and soy products.

1.1.1.5 Flavonols

Flavonols also have antioxidant and anti-inflammatory properties that cause to prohibit chronic diseases. Flavonols (quercetin, myricetin and kaempferol) which is the sub-group of flavonoids. Some fruits (like apple and berry, etc.) and vegetables (like the bean, onion, leeks, broccoli, etc.) have flavanols.

Quercetin is extensively found on earth. It has taken place in various fruits and vegetables, additionally in grains, seeds, and leaves. Further, it can be utilized in beverages, some foods, and supplements as an ingredient. However, when the health effects of quercetin consumption were evaluated by the European Food Safety Authority, no cause-and-effect relationships were validated for its physiological effects on human health or diseases. The benefits of this material to human health are still investigated. However, further research and education have been required (Bonoli, Marconi, & Caboni, 2004).

Kaempferol may contribute to the increase of antioxidant defense against free radicals that allow cancer to progress in the body. Various studies have shown the benefits of kaempferol in chronic diseases, exclusively in diminishing the risk of cancer. Kaempferol helps to prevent the development of cancer in the body by strengthening its antioxidant defense against free radicals. The growth of cancer cells and angiogenesis is importantly inhibited by kaempferol. It leads to the apoptosis of cancer cells. Contrarily, it can behave like a protector by ensuring the viability of normal cells. (Chen, & Charlie-Chen, 2013).

1.1.1.6 Flavanols

Flavanols contain three main types: monomers (most known as catechins), dimers, and polymers. These compounds are commonly taken place in some vegetables and fruits like fava beans, berry, apple, grape, and additionally in red wine and cocoa. Flavanols can be useful for helping symptoms of the exhausted syndrome and are also related to cardiac and neurology. Catechin and epicatechin are known as flavonoids. Catechin is trans-isomer and and epicatechin is cis- isomer. Each one has two stereoisomers; (+/-) catechin, and (+/-) epicatechin. In plants, mostly found isomers are (+) catechin and (-) epicatechin (Colomer, Sarrats, Lupu, & Puig, 2017). Two types of these flavonoids with strong antioxidant properties have been reported to have various physiological functions, such as anti-cancer, anti-virus and weight loss functions (Li, Zhang, & Sun, 2019).

1.1.2 Phenolic Acids

Phenolic acids are a simple group of plant phenolic compounds because of have one carboxylic acid functionality. Phenolic acids, which are not generally found in free form, are classified into two groups as hydroxycinnamic acids and hydroxybenzoic acids which are derived from cinnamic acids and benzoic acids, respectively. Caffeic acid, p-coumaric acid, chlorogenic acid, and ferulic acid are known as the most common cinnamic acid derivatives. Ellagic acid, gallic acid, p-hydroxybenzoic acid, protocatechuic acid, and syringic acid can be given as benzoic acid derivatives. These

hydroxybenzoic acids are generally found in onions and reddish fruits, etc. (Khang, Dung, Elzaawely, & Xuan, 2016).

1.1.3 Stilbenoid

According to the four large groups of polyphenolic compounds, stilbenoid belongs to the other polyphenolic compounds. Stilbenoid, a hydroxylated derivative of stilbene, is a secondary metabolite in plants that have many natural polyphenols (Hu et al., 2018). These compounds are of great interest to biologists and chemists because of their broad bioactivities such as anti-inflammatory, antioxidant, antitumor, antibacterial, neuroprotective properties, and possible therapeutic importance for chronic diseases (Leláková et al., 2019). These bioactive properties of stilbenoid are particularly related to trans-resveratrol, so its best-known member is trans-resveratrol.

1.1.3.1 Resveratrol

Resveratrol is a compound belonging to the stilbenoid group of polyphenols, consisting of two phenol rings linked by an ethylene bridge. Resveratrol is also a phytoalexin, a protective antibiotic that plants produce under stress. Phytoalexin is what helps plants recover from fungal attacks, ultraviolet radiation, and other threatening conditions. It is also found in products such as wine and juice that are made from red grapes and different Resveratrol is often consumed as a dietary supplement due to its antioxidant and anti-inflammatory properties. It is also marketed as an anti-aging supplement based on findings that it extends the lifespan of yeast cells. Resveratrol is used in inflammation, vascular, heart and liver diseases. Its use in the treatment of cancer has recently become an area of interest (Viñas, Campillo, Hernández-Pérez, & Hernández-Córdoba, 2008). Resveratrol is synthesized in fruit grains, fruit peel and lignified plant tissues. The resveratrol concentration in the pericarp of the grains is higher compared to the pulp. Is is found in the range of 0.9-8.7 mg/L in red wines and below 1 mg/L in white wines (Łukasz, Świeca, & Gawlik-Dziki, 2017).

1.2 Foods Rich in Polyphenolic Compounds

Many plant-derived foods and beverages include polyphenols as antioxidants. These compounds may have importance to prevent some chronic diseases like diabetes and cancer (Scalbert et al., 2005; Neveu et al., 2010). There have been many polyphenolic compounds with various kinds of structures in plants and food with more than 500 types of organic molecules. The heterogeneity in chemical structures of polyphenolic compounds affect the bioavailability and they have been active on health with respect to various kinds of functions and biological effects (Manach et al., 2005; Loke et al., 2008). The richest sources of these beneficial compounds in foods and beverages (fruits, vegetables, legumes, grains, nuts and seeds, herbs nd species) and others (black tea, green tea, coffee, wine, and dark chocolate, etc.) have been published (Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010).

1.3 Separation and Determination of Polyphenolic Compounds

At first, the quantitative analysis of polyphenols in plants was performed with the colorimetric method found by Folin-Ciocalteu (Hoff & Singleton, 1977). In the extraction of phenolic compounds from real sample matrix, single solvent like water, methanol or binary solvent system like water: methanol or water: THF were used as given in literatue. Various chromatographic such as thin layer chromatography, capillary electrophoresis, capillary zone electrophoresis, high performance liquid chromatography, and gas chromatography and spectroscopic methods such as nuclear magnetic resonance have been employed for identification and determination of phenolic compounds (Wong-Paz, et al., 2015; Alhakmani, Kumar, & Khan, 2013). In quantification of these compounds by gas chromatography, derivatization process is required, however, derivatization is not needed in LC methods. Angerosa and coauthors detected phenolic compounds after derivatization using a gas chromatography-mass spectrophotometer (Angerosa, d'Alessandro, Konstantinou & Di Giacinto, 1995). Many studies concerning liquid-liquid extraction and solid-phase extraction for separation and preconcentration of these compounds have been given (Pirisi, et al., 2000; Wong-Paz et al., 2015). However more recently, the separation

and determination of phenolic compounds have been performed by the solid-phase microextraction (SPME) technique.

1.4 Solid Phase Microextraction (SPME) Method

In 1989, Pawliszyn developed SPME that is an extraction and enrichment technique. In this extraction technique, the adsorbent material is coated over fiber that is made up of fused silica or metal center like copper and stainless-steel wire, and the coating thickness is ranged approximately from 7 to 100 μm. SPME is based on the partitioning of the analytes between the coating material on fiber and the sample. SPME method has different modes; direct immersion (DI-SPME) in which fiber is dipped into the sample solution, hollow fiber (HF-SPME) in which fiber is protected from the sample matrix, and headspace (HS-SPME) in which fiber is exposed to volatiles taken place above the sample for a certain time (Figure 1.1). In SPME, analytes are extracted from sample and preconcentrated on fiber surface, and then analytes are desorbed from the fiber surface (Rocío-Bautısta, Pacheco-Fernández, Pasá, & Pino, 2016). In the determination of volatile, semi-volatile, and non-volatile analytes, samples in the gas or liquid phase can be studied in the SPME method with DI- or HS-mode, and solid samples can be used after dissolving in water or extracting to the gas phase in the SPME method with DI- or HS-mode (Spietelun, Marcinkowski, De la Guardia, & Namieśnik, 2013).

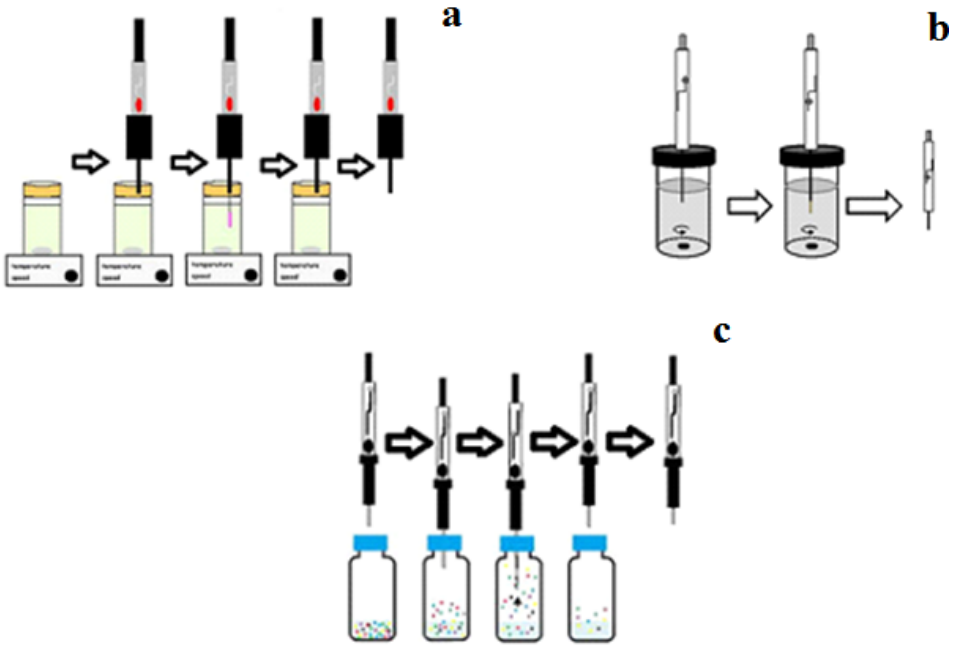


Figure 1.1 a) DI-SPME, b) HF-SPME, c) HS-SPME (Mehrdiba, Piriyaei, & Kanaani, 2016)

SPME method has advantages in terms of easiness, high preconcentration factor, the ability for automation, less sample volume, reusability, and environmental-friendly over the other traditional extraction techniques (Souza-Silva et al., 2015). It can be reported that SPME is a solvent-free technique when it is coupled to gas chromatography or a small volume of organic solvents can be used when it is coupled to liquid chromatography. Recently, new coating materials have been planned and developed for providing efficient extraction efficacy, powerful selectivity, high thermal, chemical, and mechanical stability.

1.5 Coating Materials in SPME

In SPME, the preparation of coated fiber with high extraction affinity is one of the most important steps of this technique application. In the SPME method, less volume of sample solution and less or no desorption solvents are required, and interference can be less because of the selectivity of coating material (Piñeiro-García et al., 2018). So, more and more new materials are used as coating materials for the preparation of SPME fibers. Materials based on the properties are divided into two types: organic and

inorganic. Firstly, organic polymer materials were produced as coating materials for SPME fibers. Fused silica fibers known as customary SPME fibers are suitable for functionalization with polymeric materials (Jiang, Huang, Cai, & Zhao, 2006). Polysiloxane was an important coating material for SPME fiber due to its high stability of thermal, good performance for film formation, and is also used as a stationary phase in gas chromatography. In addition, it can be easily derivatized or functionalized to form new coating materials (Wu & Zeng, 1990). In recent years, new coating materials such as graphene oxide, single-walled or multi-walled carbon nanotubes, ionic liquids, polymeric ionic liquids, molecularly imprinted polymers have been developed for SPME fiber. Within these, ionic liquids-based materials have been preferred to design new SPME coating materials because of their easy synthesis and their selectivities (Wang et al., 2011). In preparation of SPME fibers, sol-gel coating, electrochemical polymerization, and layer by layer in situ fiber coating can be given as some common techniques (Ciardina, & Olesik, 2003; Giardina, Ding, & Olesik, 2004).

1.5.1 Graphene Oxide

Recently, graphene is largely used in many studies due to its excellent properties like good resistance to corrosion, high chemical stability, good surface area, good hardness, and attractive thermal and mechanical properties (Figure 1.2) (Rezaei, Akhavan, Hashemi, & Shamsara, 2016). Graphene oxide that is obtained from graphite oxidation can be also modified by using electrochemical reduction, thermal or chemical methods. On the other hand, the usage of graphene oxide is increasing because of its excellent mechanical properties and chemical variability (Ho, Zhang, Hantao, & Anderson, 2014). Finally, graphene oxide is preferred in analytical chemistry because of its functionalization, high reactivity, and large applicability to design new materials (Piñeiro-García et al., 2018).

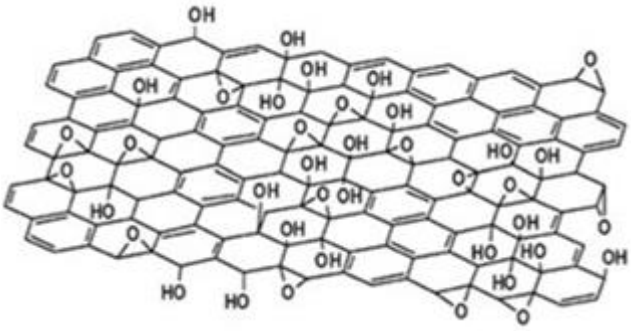


Figure 1.2 Structure of graphene oxide (Wang et al., 2013)

1.5.2 Montmorillonite

The clay minerals are also attractive alternatives to the other environmental materials and by enhancing their performance, are used in various areas such as catalysis, food additive, antibacterial function, and sorbent (Zhou et al., 2019). Montmorillonite like other clays is inexpensive and has non-toxic properties, a distinct layered structure, considerable surface areas, mechanical- chemical stabilities, and elicits a strong ability to ion-exchanged (Hao et al., 2019). It consists of SiO_2 units and by connecting via the 3 oxygen atoms at the corners of these units a 2-dimensional is formed (Figure 1.3). Because of the characteristics of cation exchange capacity, sorption capacity, high porosity, and large surface area, montmorillonite composite research is still receiving a great deal of attention (Silva et al., 2019).

Recently, novel nanocomposites/composites prepared with modified montmorillonite clay with surfactants or ionic liquids have been used for the adsorption of organic compounds (Krishna Kumar et al., 2012). Ion with organic cation has shown excellent prospect in adsorb organic compounds from aqueous solution and significant development in the use and application of montmorillonite in this field is seen in recent times.

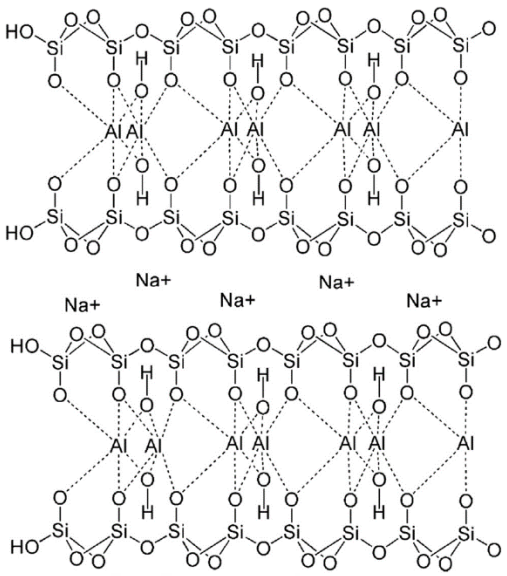


Figure 1.3 Structure of montmorillonite (Salavati-Niasari, 2007)

1.5.3 Ionic liquids (ILs)

ILs are composed of big asymmetric organic cations and anions which can be organic and inorganic. They have some advantages such as high thermal stability, low volatility, non-flammability, less toxicity and miscible with hydrophilic and hydrophobic solvents. ILs have been promoted as designer solvents because the cation-anion combination can be tuned and designed according to its potential applications (Figure 1.4) (Raja Shahrom, Nordin, & Devi Wilfred, 2019). Due to these properties, ILs have been used in many areas (Souza-Silva, et al., 2015). In this sense, researches have been conducted to progress novel coatings for SPME fiber comprised of ILs and their application in analytical chemistry. The selectivity of ILs has been enlarged by functionalization with some organic groups. Therefore, ILs have been used effectively as coating materials for SPME fiber by supplying preferable performances with respect to commercial fiber coatings (Gionfriddo et al., 2018).

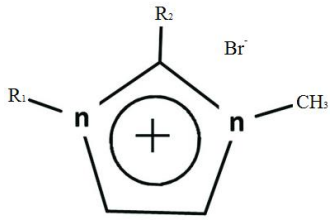


Figure 1.4 A general scheme of imidazolium based ILs (Kumar Singh et al., 2018)

1.6 Gas Chromatography- Mass Spectrometry (GC-MS)

Detection and quantification of volatile organic molecules in a sample is investigated with a gas chromatography-mass spectrometer (GC-MS). GC separates the components in a sample mixture and MS identifies the component with the help of fragmentation patterns of mass spectral of the related compound (Yang et al., 2017). In GC-MS, firstly components in the sample mixture are separated by GC, these components are transferred to the ionization chamber in MS and components are ionized, ions are separated in MS analyzer, ions are detected by electron multiplier and finally, data are processed and displayed by a computer system (Mani, Kalpana, Patil, & Dayal, 2017). Many kinds of components in the sample are entrained along the column with the aid of carrier gas. These components are separated depending on their volatility and their partitioning between the stationary phase (coated material on column) and a mobile phase (carrier gas). Stationary phases used to prepare GC columns are specific and selective for separation of molecules found in sample matrix (Yan-Fen et al., 2019). In this way, GC-MS equipped with powerful data systems is highly useful in food analysis and food science due to the sensitivity of GC-MS.

Recently, the researchers have been focused on the development of SPME techniques with GC-MS due to its selective, rapid, efficient, economical, simple, and solvent-free. Volatile and non-volatile compounds at trace amounts in many kinds of sample matrices can be concentrated in SPME methods. In this study, non-volatile phenolic compounds are analyzed with SPME coupled with GC-MS due to possible main advantages such as increased sensitivity, no solvent usage (Bianchi et al., 2017). However, these compounds need chemical derivatization before GC-MS analysis. By the derivatization step, non-volatile compounds are converted into derivatives that are volatile and have thermal stability. This step can be applied during the sample preparation step, after the SPME method on fiber surface prior to GC analysis, or at the desorption step in the injection port of GC. On-fiber derivatization method, SPME fiber can be exposed to the derivatization reagent after the SPME method or before extraction in the sample solution. Generally, SPME method is applied to sample

solution and later derivatization reagent vapor was adsorbed by analyte on fiber surface (Viñas, Campillo, Martínez-Castillo, & Hernández-Córdoba, 2009).

1.7 Liquid Chromatography (LC)

LC is the most used chromatographic method as the analytical separation technique that is used to separate a mixture of compounds with the purpose of identification, quantification, or purification of the individual components in the mixture. LC's advantages are its sensitivity, accurate quantitative determination, and its suitability for the separation of non-volatile or thermally decomposing compounds (Li et al., 2020). In the separation of components in a mixture, a mobile phase has significant importance. The types of components and their volumes are effective in the separation of target molecules in the sample medium. The main purpose in LC, the elution of each compound must be achieved in a reasonable time with high resolution. Normal phase or reversed-phase chromatography can be applied by comparing the polarity of stationary phase with the mobile phase (Kotapati, & D.Bares, 2018). Reversed-phase LC (RP-LC) is the most preferred type. Hydrophobic molecules and compounds having different organic functionality are better separated by RP-phase LC. In these process, van der Waals forces and hydrophobic interactions take place between RP column and suitable mobile phases and analytes (Canene-Adams, 2013).

Many semi- or non-volatile organic compounds are best separated by LC. Thus, it would be advantageous the SPME technique with LC such as providing easy sample preparation and as well as high resolving power for semi- and non-volatile analytes. As known, the SPME technique has two steps; the first is, extracting the analytes from a sample and the second is, elution of these analytes from the surface of SPME fiber. For SPME-LC coupling, the extraction procedure is like that used for GC analysis. The main difference between SPME-LC and SPME-GC is in the desorption procedure. In GC analysis, the adsorbed analytes are desorbed from the surface of SPME fiber by heating. However, in LC analysis non-volatile compounds cannot be desorbed from SPME fiber at high temperature. So, a desorption solution is an alternative way for this

purpose. The analytes are desorbed with a minimum amount of desorption solution and injected directly into the LC column (Hu, Hu, & Li, 2007).

1.8 Related Studies for Analysis of Phenolic Compounds by SPME Method

In recent years, there are numerous articles about the analysis of phenolic compounds in foods. Several papers about preconcentration and determination of these compounds are given below:

Citova and coworkers developed a derivatization method using alkyl chloroformate campounds for phenolic acids (caffeic, ferulic, gallic, p-coumaric, protocatechuic, syringic, vanillic) in aquaeous solution including polar solvents and pyridine (Citova, Sladkovský, & Solich, 2006). In this study, extraction parameters were optimized using different commercial fibers (polyacrylate, polydimethylsiloxane, carboxen/polydimethylsiloxane and polydimethylsiloxane/ divinylbenzene). Methyl chloroformate as derivatization reagent and the polyacrylate as the SPME fiber were selected after optimization experimental conditions. The quantitation limits of the studied phenolic acids were 0.02 to 0.2 $\mu g\ mL^{-1}$. The developed derivatization method increases the sensitivity of the proposed SPME coupled to GC method ten times higher than the original GC method.

Trans- and cis-resveratrols were determined in fruit juices, wine and musts with SPME and stir bar sorptive extraction (SBSE) methods coupled to LC with floresance detector (Viñas, Campillo, Hernández-Pérez, & Hernández-Córdoba, 2008). The efficient extraction efficiency was obtained with a carbowax template resin coated SPME fiber in SPME and polydimethylsiloxane coated stir bar in SBSE. Under optimized conditions, the linear range and limit of detecitons of cis isomer of resveratrol was a little bit better than its trans isomer.

Determination of resveratrol in GC is required derivatization due to its polar character. Cai and coauthors (2009) developed a derivatization method on fiber after SPME method before multi dimensional GC-MS system using silyl derivative

reagents. Under optimal conditions, the proposed method has very large linearity, good recovery, low limit of detection in wine samples.

To determine trans-resveratrol in wine samples, solid phase extraction method using mix-mode sorbent was developed and derivatization by acetylation was achieved using silylation reagent before GC-MS analysis. The method was linear up to 2.5 μg mL^{-1}. Trans-resveratrol levels in wine samples were ranged from 3.4 to 1810 ng mL^{-1} and cis-resveratrol was also detected n these samples (Montes , García-López, Rodríguez, & Cela, 2010).

Eight phenolic compounds were first extracted with the SPME method using polystyrene-divinylbenzene-polyacrylonitrile coating and analyzed by liquid chromatography-mass spectrometry method. Recovery above 80%, low limit of detections (0.2-3 ng mL^{-1}), good precision (4-8% relative standard deviation), and large linearity (1-1000 ng mL^{-1}) were obtained. The developed method was applied to grape, berry, and wine samples (Mirnaghi, Mousavi, Rocha, & Pawliszyn, 2013).

For the determination of gallic acid and protocatechuic acid in wine samples with LC, molecularly imprinted polymers and non-imprinted polymers were developed and used as a solid-phase extraction sorbent. The synthesized both molecularly imprinted polymers recognized only protocatechuic acid and molecular imprinted polymer for gallic acid recognized its target molecule. The linearity was between 10 and 70 μg mL^{-1} for gallic acid and 0.1 μg mL^{-1} and 4.5 μg mL^{-1} for protocatechuic acid. The limit of detections was less than 0.4 μg mL^{-1} (Denderz & Lehotay, 2014).

Four different phenolic acids were determined by LC with diode array detector (DAD) after SPME method using polydimethylsiloxane- divinylbenzene coated commercial fiber in various fruits and vegetable samples. The effect of experimental conditions for extraction method and chromatographic analysis were investigated. Acetonitrile-phosphate buffer was chosen as elution solvent. The developed method presented a low limit of detections and good within-day and between-day precisions (Aresta & Zambonin, 2016).

Trace amount of phenolic acids were analyzed by LC-DAD after SPME method with multiple monolithic fiber in fruit juice samples. The effect of extraction conditions were optimized. The linearity was in the range of 1.0–200 μg L^{-1} for all phenolic acids and the detection limits were llower than 0.57 μg L^{-1} (Pei & Huang, 2016).

Molecular imprinted polyer was synthesized and used as a coating material for SPME fiber in the detection of phenolic acids using SPME coupled to LC. To synthesize polymeric ionic liquid, 1-ally-3-vinyl imidazolium chloride as a functional monomer, 3, 4-dihydroxybenzene propanoic acid as template molecule, and ethylene dimethacrylate as cross-linker were used. Under optimal conditions, phenolic acids were determined in real beer and fruit juice samples. The detection limits of phenolic acids were 0.024–0.24 μg L^{-1} in fruit juice and 0.011–0.052 μg L^{-1} in beer samples (Chen & Huang, 2017).

In this study, phenolic acids were extracted with microextraction by packed sorbent using C18 and micro liquid-liquid microextraction and analyzed by GC-MS after trimethylsilyl derivatization. The plasma samples were prepared using miaturized sample pretreatment techniques (Bustamante et al., 2017).

In this work, SPME combined with LC using DAD detector was proposed for detection of trans-resveratrol in real grape juices, spirits and wines. The experimental parameters including extraction and desorption were studied. The amounts of trans-resveratrol in the studied samples were between 0.007 and 4.486 μg mL^{-1} (Aresta, Cotugno, Massari, & Zambonin, 2018).

1.9 Purpose of the Study

Because the progressing of analytical methodologies for the determination of phenolic compounds in food products is getting more and more important, we aimed the preconcentration of phenolic compounds by using novel coating materials for

preparation of fiber to use in solid phase microextraction method and determination of them by GC-MS and LC techniques in this study. The steps done:

(1) Ionic liquids based on methylimidazolium cations with different anions such as 1-(3-aminopropyl)-3-methylimidazolium bromide (APMI-Br), 1-(3-aminopropyl)-3-methylimidazolium tetrafluoroborate (APMI-BF_4) and 1-(3-aminopropyl)-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (APMI-TFSI) were synthesized and characterized.

(2) Ionic liquids based on benzylimidazolium cations with different anions such as 1-(3-aminopropyl)-3-benzylimidazolium bromide (APBI-Br), 1-(3-aminopropyl)-3-benzylimidazolium tetrafluoroborate (APBI-BF_4) and 1-(3-aminopropyl)-3-benzylimidazolium bis(trifluoromethylsulfonyl)imide (APBI-TFSI) were synthesized and characterized.

(3) Coating materials (graphene oxide-ionic liquid and montmorillonite-ionic liquid) for SPME fibers were prepared. Structure and thermal properties of coating materials and the prepared fibers (FTIR, DTA, XRD, SEM were characterized.

(4) Phenolic compounds by GC-MS and LC were separated.

(5) SPME-GC-MS and SPME-LC methods were optimized.

(6) Analytical parameters for SPME-GC-MS method using graphene oxide-ionic liquid coated fiber with high extraction efficiency were studied.

(7) Analytical parameters for SPME followed by GC-MS and SPME followed by LC methods using montmorillonite-ionic liquid fiber with high extraction efficiency were studied.

(8) Phenolic compounds were analyzed in wines by SPME-GC-MS method using selective graphene oxide-ionic liquid coated fiber.

(9) Solid phase microextraction combined to gas chromatography-mass spectrometry and solid phase microextraction combined to liquid chromatography methods were used for extraction of phenolic compounds from fruit juices with the use of selective montmorillonite-ionic liquid coated fiber.

(10) Comparison of prepared SPME fibers with commercial ones.

CHAPTER TWO
EXPERIMENTAL METHODS AND MATERIALS

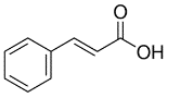
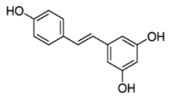
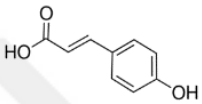
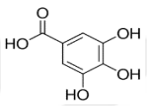
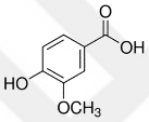
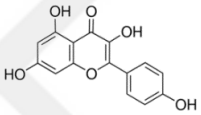
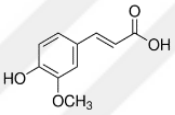
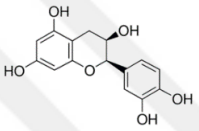
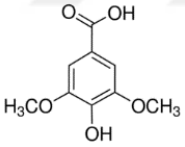
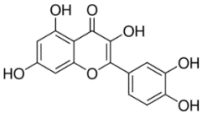
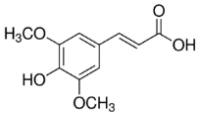
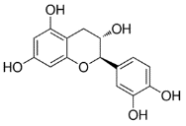
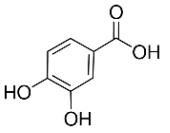
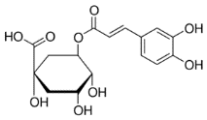
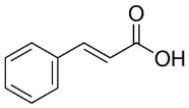
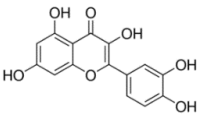
2.1 Chemicals and Materials

Phenolic compounds (kaempferol (Ka), ferulic acid (FA), quercetin (Q), syringic acid (SA), rutin (R), caffeic acid (CA), resveratrol (Res), p-coumaric acid (pCA), gallic acid (GA), protocatechic acid (PrCA), chlorogenic acid (CGA), cinnamic acid (CnA), vanillic acid (VA), sinapinic acid (SnA), epicatechin (EC), catechin (C), methanol and acetonitrile (LC purity), N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), ethyltrimethylsilane (ETMS), trimethylchlorosilane (TMCS), 1-methylimidazole, 3-bromopropylamine hydrobromide, bis (trifluoromethane) sulfonimide lithium (LiTFSI), ammonium tetrafluoroborate (NH_4BF_4) and graphene oxide (GO) (2 mg/mL, dispersed in water) were purchased from Alfa Aesar, Merck, or Sigma-Aldrich. Clay as montmorillonite (KSF) (20-40 m^2/g surface area) and cross linker as MDEU (modified dihydroxy ethylene urea) were supplied (Fluka and Hunstman). Montmorillonite clay is composed of SiO_2 (55.0%), Al_2O_3 (18.0%), sulfate (5.0%), Fe_2O_3 (4.0%), CaO (3.0%), MgO (3.0%), K_2O (1.5%), Na_2O (<0, 5). In Table 2.1, structures and pKa and Log P values of studied compounds are summarized. Amber glass vial with cap and polytetrafluoroethylene/silicone septum (20 mL), manual SPME fiber holder, commercial SPME fibers (carbowax/polyethylene glycol (CW/PEG, 60 µm), divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm), carboxen/ polydimethylsiloxane (CAR/PDMS, 85 µm) and polyacrylate (PA, 85 µm)) were supplied from Supelco. Five µL Hamilton micro syringe was used to prepare lab-made fibers in the laboratory. Stainless steel wire having an outer diameter as300 µm was supplied from the local market for the preparation of lab-made SPME fiber.

Standard solutions of phenolic compounds as 1 mg mL^{-1} in methanol were prepared and kept in refrigerator. Intermediate mix phenolic compounds solution was prepared in methanol from the stock standard solutions of each analyte. In the optimization studies, aqueous solutions including all phenolic compounds at a concentration of 200

mg L^{-1}were prepared weekly with distilled water. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) at 1 mol L^{-1} was used to prepare buffer solution at pH 8. Synthetic wine solution was prepared at pH 3.5 with tartaric acid and its alcohol content was adjusted to 12% (v/v) with ethanol. These prepared test solutions were used in SPME method for optimization and analytical performance studies.

Table 2.1 Chemical properties of phenolic compounds (Al Harthi et al., 2014)

Compound	Structure [k]	Log P	pK_a	Compound	Structure [k]	Log P	pK_a
CnA		2.14[a], 2.41[b]	4.51[a], 4.44[b]	Res		3.4[a], 3.06[e]	8.49[a], 8.8[f]
pCA		1.83[a], 1.46[c]	4[a],4.64[c]	GA		0.72[a], 052[g]	3.94[a], 4.4[h]
VA		1.17[a]	4.16[a]	Ka		2.46[a], 3.11[j]	6.44[a]
FA		1.61[a], 1.51[c]	3.27[a], 4.58[c]	EC		1.8[a]	8.72[j]
SA		1.01[b], 1.04[c]	3.93[b], 4.34[c]	Q		2.16[a], 1.82[i]	6.38[a]
SnA		1.52[a]	3.61[a]	C		1.8[a]	8.64[h]
PrCA		1.02[a]	4.16[a]	CGA		-0.27[a]	3.33[a]
CA		1.53[a]	3.64[a], 4.77[d]	R		-0.87[a]	6.43[a]

2.2 Apparatus

Fourier-transform infrared (FTIR) spectrometer (Perkin Elmer Spectrum 100), x-ray diffractometer (XRD) (Philips X'Pert PROBE), thermogravimetry/differential thermal analyzer (TG/DTA) (Perkin Elmer Diamond), scanning electron microscope-energy dispersive x-ray (SEM-EDS) spectrometer (Zeiss Sigma 500) were used in the characterization of synthesized ionic liquids, the coating materials of GO-IL and MMT-IL and the lab-made SPME fibers. X-ray diffraction (XRD) spectra (Cu Kα source, $2\theta\ s^{-1}$) in the range of 3º - 15º or 10º - 60º were obtained. Thermogravimetric curves were obtained in the range of 30 ºC - 450 ºC by a heating rate of 10 ºC min^{-1} under N_2 flow. Ultrapure water used in all experiments. Nuve NF 200 model centrifuge was used for centrifugation and Mettler Toledo model pH meter was used for pH studies.

2.3 Chromatographic Methods

LC-diode array detector (DAD) (Thermo Scientific Dionex Ultimate 3000) and GC-MS (Thermo Scientific Trace 1300) were used for analyte determination. Capillary column (TG-5MS) (30 m x 0.25 mm i.d., 0.25 μm film thickness) was used for GC chromatographic analysis. High purity He gas was used as carrier gas. The flow rate was 1.2 mL min^{-1}. Injection in splitless mode was selected. Electron impact (EI) was performed at 70 eV. The ion source temperature was 280 °C and mass transfer line temperature was 250 °C. The gradient temperature program was as follows: 80 °C (3 min held), 220 °C by heating rate of 10 °C min^{-1} (2 min held) and 280 °C by heating rate of 20 °C min^{-1} (2 min held). Selected ion monitoring (SIM) was used for data acquisition. In Table 2.2, selected qualification and quantification ions and retention times (t_R) of analyte derivatives were listed. In Figures 2.1 and 2.2, the phenolic compounds GC chromatograms were given.

In LC analysis, chromatographic separation was performed with a C18 column (150 mm × 4.6 mm, 5 μm particle size) at 30 °C. The 2%HAc: MeOH: ACN, 70:20:10 (v/v) was applied as mobile phase in isocratic condition.The injection volume and flow rate

were 20 μL and 1 mL min^{-1}, respectively. The detections were performed at 320 nm for ferulic acid, chlorogenic acid, syringic acid, gallic acid, p-coumaric acid, caffeic acid, cinnamic acid, protocatechuic acid, sinapinic acid, and resveratrol, at 370 nm for quercetin and kaempferol, at 254 nm for vanillic acid and rutin and at 278 nm for catechin and epicatechin (Fig. 2.3).

Table 2.2 Retention times, selected quantification, and identification ions of phenolic compounds (Stanoeva, Peneva, Stefova, & Gjamovski, 2019)

Compounds	t_R (min)	Qualifying ions (m/z)	Quantifying ions (m/z)
CnA	4.46	131, 145,161, 205, 220	131
pCA	6.02	219, 245, 293, 308	219
VA	7.25	149, 165, 223, 312	149
FA	8.65	219, 249, 323, 338,	249
SA	9.32	297, 312, 327, 342	327
SnA	11.01	249, 338, 353, 368	353
PrCA	12.14	193, 355, 370	193
CA	13.07	191, 219, 381, 396	219
Res	14.85	179, 267, 429, 444	267
GA	15.82	147, 281, 458	281
Ka	16.43	272, 487, 559, 574	487
EC	16.81	147, 267, 355, 368, 650	368
Q	17.34	559, 574, 647	647
C	17.71	179, 267, 355, 368, 650	368
CGA	19.14	345, 397, 419, 786	419
R	19.82	301, 465	465

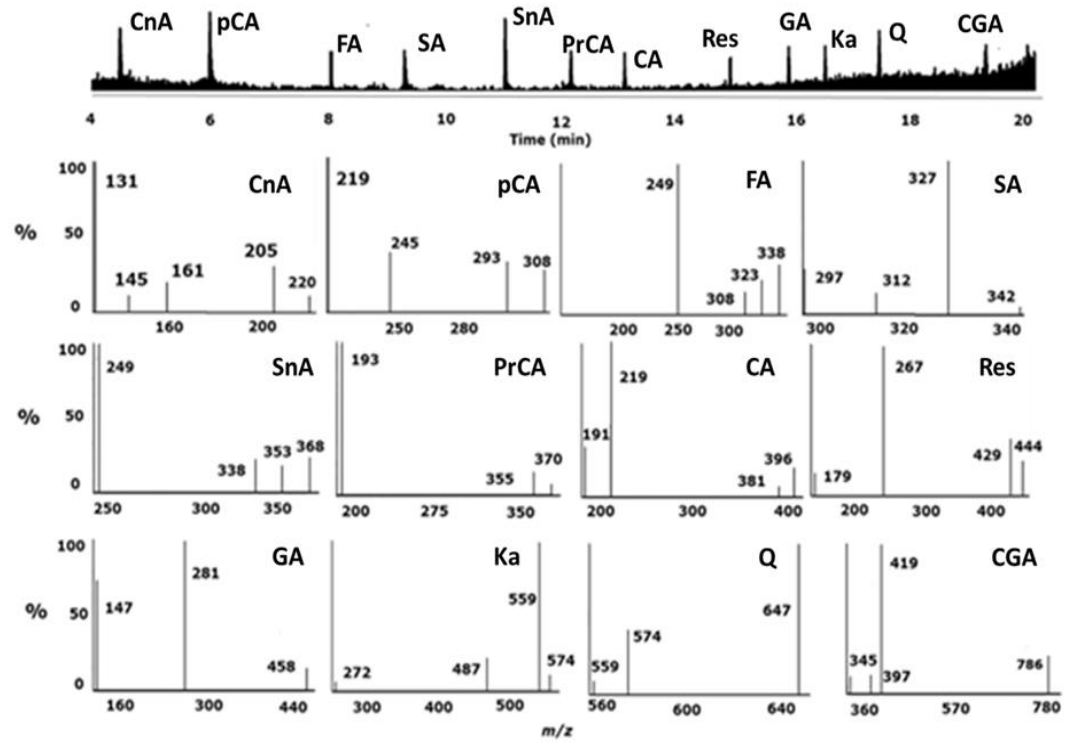


Figure 2.1. Chromatogram of phenolic compounds standards and MS spectra of phenolic compounds by the proposed method

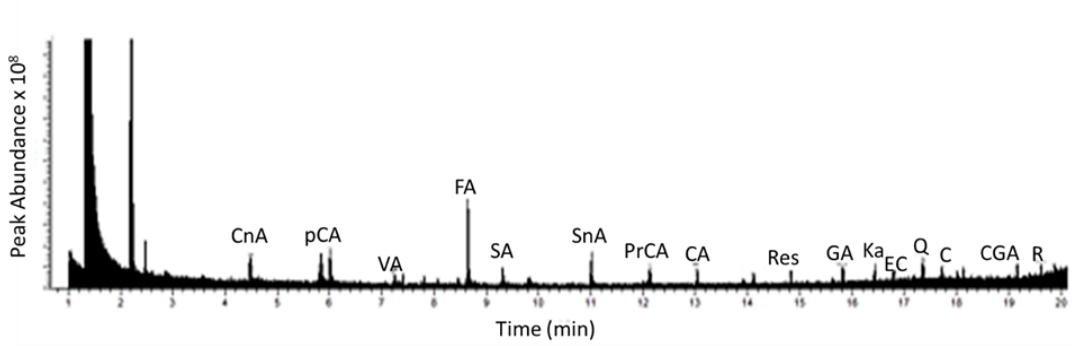


Figure 2.2. Chromatogram of phenolic compounds standards by the proposed method

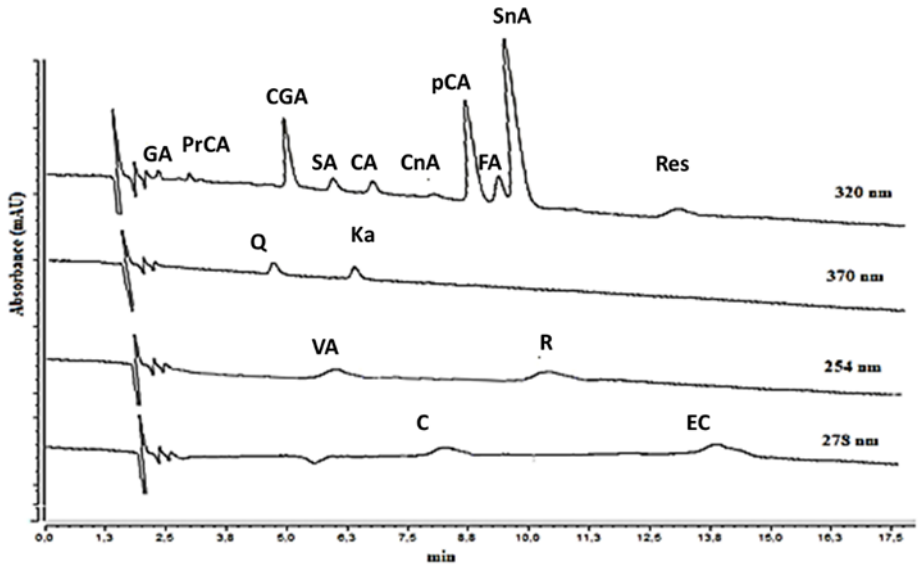


Figure 2.3 Chromatogram of phenolic compounds standards by the proposed method

2.4 Samples

The analyzed samples including 2 red wines (Okuzgozu), 2 white wines (Ancyra, and Chardonnay) and 4 fruit wines were bought from local market in Izmir, Turkey. All samples were produced in Turkey (Canakkale, Usak, Manisa, Kırsehir, and Şirince) in 2013 and 2017 from different grapes (Chardonnay, narince, okuzgozu) and other fruits (black mulberry, blueberry, melon, and red plum). The alcohol content was in the range of 12%-13.8% for the studied wines. The bottles of wine were stored at 4 ºC and protected from light before analysis. Before applying the SPME method, 5 mL of wine samples were taken, their pH is adjusted to 8 and diluted to 20 mL with ultrapure water.

Fresh fruit samples (apple, pear, grapefruit, mandarin, quince, pomegranate, and orange) were bought from the market in İzmir, Turkey. After washing, cleaning, and removing seeds, the fruits were squeezed with a manual juicer. In next step, fruit juices were filtered using a cloth of cotton to separate pulp and foam. Then, these samples were filtered using Hydrophilic PVDF filter cartridges as 0.45 µm (Millipore). Before applying the SPME method, the pH of the 5 mL sample filtrate was adjusted to 4 using 0.1 mol L^{-1} HCl and was diluted to 20 mL using ultrapure water.

2.5 Synthesis of Ionic Liquids

In the study, APMI-Br, APMI-BF_4 and APMI-TFSI, APBI-Br, APBI-BF_4 and APBI-TFSI were synthesized (Yang, Shan, Li, Han, Zhang, & Niu, 2009; Tashakkori et al., 2019).

For this, 5 mmol of 1-methyl/benzimidazole and 5 mmol of 3-bromopropylamine hydrobromide were dissolved using ethanol as 15 mL. The final solution was stirred and refluxed for 24 hours at 80 °C under N_2 flow. The resulting product was crystallized using ethyl acetate in ice bath. The ethanol was then evaporated on the rotary evaporator and the APMI-Br/APBI-Br ere dried at 50 °C under vacuum for 24 h.

By reacting APMI-Br with LiTFSI and NH_4BF_4 salts, ionic liquids containing BF_4^- and $TFSI^-$ anions were obtained. APMI-Br and equimolar salts were mixed in 10 mL of methanol for 24 hours at room temperature. The resulting mixture was washed several times with a few mL of distilled water to remove free bromide and then filtered. Then the methanol was evaporated on the rotary evaporator and the APMI-TFSI/APMI-BF_4 was dried in vacuum oven at 50 °C. With similar procedure, APBI-BF_4 and APBI-TFSI ionic liquids were prepared.

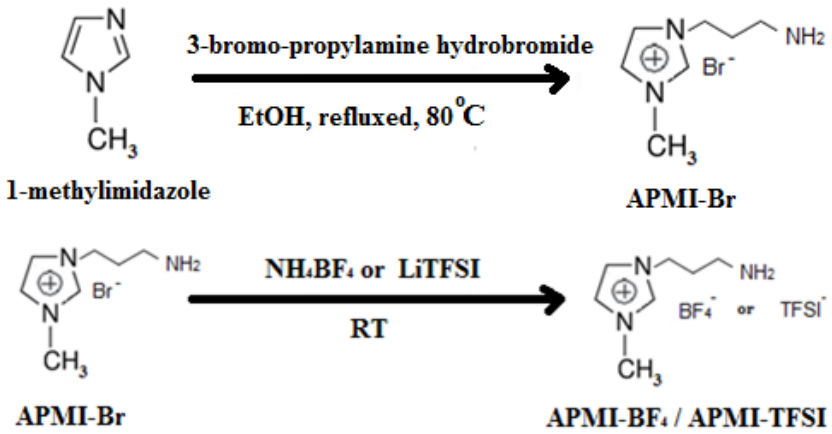


Figure 2.4 Synthesis of APMI-Br, APMI-BF_4 and APMI-TFSI

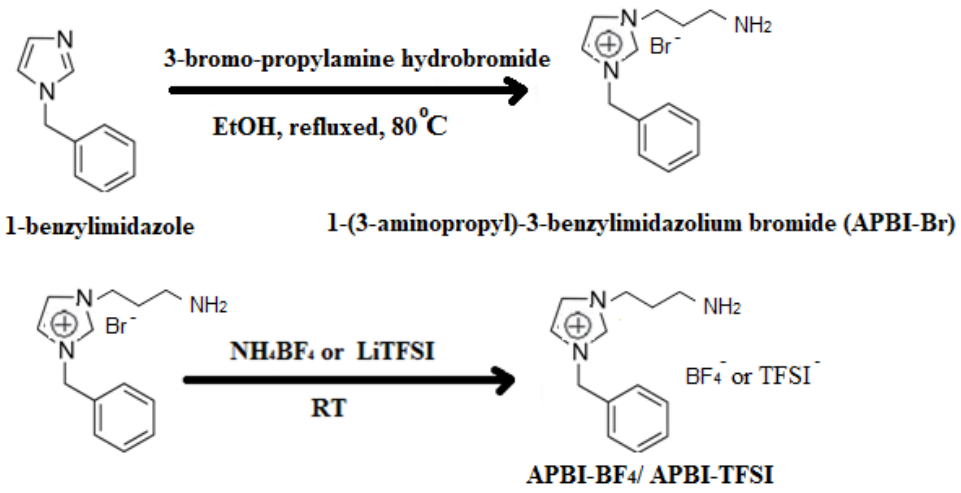


Figure 2.5 Synthesis of APBI-Br, APBI-BF_4 and APBI-TFSI

2.6 Preparation of Lab-made SPME Fibers

2.6.1 Surface Functionalization of Stainless-steel Wire

Firstly, the 1.5 cm of tip of the 15 cm long stainless-steel wire was washed with acetone, ethanol, and deionized water to clean the surface of the wire. After the wire was dried at room temperature, the cleaned end of the wire was immersed in a solution containing equal volumes of aqua regia and water solution including an equal volume from both for 30 min. In the next step, the tip of the wire was first rinsed with water and dried again. Later, it was inserted into sodium hydroxide solution (2 M) after the etching step. The basic treated tip of stainless-steel wire was washed with deionized water and then put into 0.01 M hydrochloric acid for 15 min to be neutral, washed with deionized water again, and dried in air at room temperature for 5 min. Finally, the modified wire was dried for 30 min at 150 ºC under N_2 flow.

2.6.2 Preparation of SPME Fiber with Graphene oxide-Ionic liquid

After etching, the end of the hydrolyzed steel wire was immersed in the MDEU cross linker for 1 hour, then in the slurry of GO for 30 minutes. For sufficient coating, this step was occured 3 times in the same way. The GO coated fiber was dipped

vertically in 0.02 g of APMI-BF_4/APMI-TFSI in methanol and incubated for 2 hours at room temperature. For sufficient coating, this step was occurred 6 times in the same way. The prepared GO-IL fibers were first conditioned at room temperature overnight and then conditioned more than 5 times in GC-MS at 175 ºC for GO-APMI-BF_4, and 250 ºC for GO-APMI-TFSI for 5 min under He atmosphere.

2.6.3 Preparation of Montmorillonite- Ionic liquid Coated SPME Fiber

KSF is in a powder form with a typical particle size of less than 2 μm and was used for the ion exchange modification with ILs. The KSF coated fiber was prepared by on fiber coating. Previously, the above modified wire put into 1 mL of MDEU crosslinking reagent in tube for 1 h. Then, the treated wire end was dipped into 1 % (w/v) KSF suspension for 6 h under room temperature. The fiber was removed and dried for 30 min at 80 ºC. These steps were repeated several times for a day for reaching homogeneous coating. Then, the KSF-coated fiber was inserted into a 5 mL of methanol solution with 0.5 g of each of APMI-Br/ APMI-BF_4/ APMI-TFSI/ APBI-Br/ APBI-BF_4/ APBI-TFSI ionic liquids for 24 h at room temperature. After this step, the lab-made SPME fibers were washed with ethanol to get rid of the excess ILs, and dried at 80 ºC under vacuum for 24 h. At the end, the fibers were conditioned at least 8 times in GC-MS for 5 min at around 200-250 ºC under He atmosphere.

2.7 Direct Immersion Solid Phase Microextraction (SPME) Method for Optimization Studies

For SPME experiments, working aqueous solutions were prepared by adding 10 μL of 200 mg L^{-1} of the standard phenolic compounds to synthetic wine samples or aqueous test solutions in 20 mL amber glass sampling vial with a silicone septum. After equilibration for 5 min, the 1 cm length of the lab-made SPME fiber was directly put into the aqueous solution at adjusted pH at suitable temperature for a certain extraction time while stirring at 400 rpm using a 1 cm long of stirring bar. Extractions were operated with a heater with a magnetic stirrer and metallic block. All determinations were performed in triplicates.

After derivatization, phenolic compounds were desorbed by injected the GO-IL coated fibers at 200 °C and KSF-IL coated fibers at 250 °C into the GC port for 5 minutes. The coated fibers were heated at 200-250 °C in another injection block of the GC for 3 minutes after desorption to remove possible deposition on the fiber.

2.8 Analysis of PCs by LC-DAD After SPME Method

The KSF-IL coated fiber was dipped into a 1.5 mL vial containing a certain volume of elution solvent and kept at a suitable temperature for a certain time in the analysis of phenolic compounds by LC following the SPME method. After the elution step, the fiber was immersed in MEOH, ACN, and deionized water for 1 min to prevent deposition on it. At the end, the fiber was dried at room temperature before next experiment.

2.9 SPME Method for Wine Samples

Wine sample solution prepared as given Section 2.4 was taken as ~20 mL, the pH was adjusted to 8 using 0.1 M NaOH solution. The ionic strength of that solution was arranged to 10% (w/v) NaCl and placed into the sample vial. The extraction was performed at 30 °C for 30 minutes by stirring at 400 rpm. After extraction, the fiber was dried under N2 atmosphere for 5 min to prevent the hydrolysis process and exposed to the vapor of derivatization reagent mixture (20 µL) containing TMCS and BSTFA at 9:1(v:v) at 60 °C for 15 min. Finally, the GO-IL coated fiber was put into the GC port, and analytes were desorbed at 175 °C or 250 °C for 5 min. To prevent accumulation on the surface of the coated fiber, it was subjected to heating in GC port at 175/ 250 °C for 3 min.

2.10 SPME Method for Fruit Juice Samples

Fruit juice sample solution prepared as given Section 2.4 was taken as ~20 m. The pH was adjusted to 4 using 0.1 M HCl solution. The ionic strength of that solution was

arranged to 10% (w/v) NaCl and placed into the sample vial. The extraction was performed at 30 °C for 45 minutes by stirring at 400 rpm. After extraction, the fiber was dried under N_2 atmosphere for 5 min, placed in headspace of a flask including 20 µL derivatizing agent (BSTFA: TMCS (9:1, v: v)) at 60 °C for 15 minutes in the derivatization step. Later, the KSF-IL coated fiber was put into GC port and analytes were desorbed at 200 °C for 5 minutes. To avoid carryover, the fiber was heated in the other GC port at 200 °C for 3 minutes.

Following the SPME method, it was immersed into a 500 µL of eluent solvent (2%HAc/MeOH/ACN, 70/20/10 (v/v)) for 10 minutes at 30 °C for the determination of phenolic compounds with the LC-DAD method. A 20 µL of eluent was inserted to the LC system for the determination of phenolic compounds. After the elution, methanol, acetonitrile, and ultrapure water was used to wash the fiber. After washing, the fiber was dried at room temperature before next usage.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1 Characterization of Coating Materials

3.1.1 FTIR Analyses

FTIR spectra of ILs are given in Figure 3.1 and 3.2. N-H, aromatic and aliphatic C-H, aromatic C=N and C=C, aliphatic C-N and C-C stretching vibrations in all synthesized ionic liquids and S=O, C-S and C-F stretching vibrations in ionic liquids with anion $TFSI^-$ were observed (Feng, Sun, Bu, & Luo, 2015). The N-H stretching vibrations at 3381- 3432 cm^{-1}, aromatic and aliphatic C-H stretch vibrations at 3149-3004 cm^{-1} and 2982- 2806 cm^{-1}, C=N stretching vibration at 1561- 1571 cm^{-1}, C=C stretch vibration at 1490- 1554 cm^{-1}, -C-N stretching vibration at 1185- 1157 cm^{-1}, -C-C stretching vibration at 1131- 1020 cm^{-1}, -C-F stretching vibration at 1451- 1457 cm^{-1}, S=O stretching vibration at 1054- 1457 cm^{-1}, and -C-S stretching vibration in the range of 741- 740 cm^{-1}were observed.

The OH stretching belonging to the hydroxyl groups in the structure of graphene oxide at 3310 cm^{-1}, the C=O stretching belonging to the carboxyl groups at 1719 cm^{-1}, the C=C stretching at 1610 cm^{-1}, the CO bending vibrations of the epoxy and alkoxy groups at 1410 and 1265 cm^{-1} were seen (Li et al., 2017). Only the GO-APMI-TSFI spectrum is given as an example in Figure 3.3. In the GO-ILs spectra, –OH stretching vibrations from GO, aliphatic CH stretching vibrations from ionic liquids, C=N stretching vibration of ionic liquid and C=C stretching vibration from GO and ionic liquid were obtained at 3372- 3345 cm^{-1}, 2923- 2841 cm^{-1}, 1557- 1555 cm^{-1} and 1452-1444 cm^{-1}, respectively.

In the KSF spectrum, OH stretching vibration at 3624 cm^{-1}, water OH stretching and HOH bending vibration at 3417 and 1630 cm^{-1}, Si-O stretching vibration at 1048, 525, 464 cm^{-1} and Al-OH bending vibration at 917 cm^{-1} were observed. N-H, aliphatic and aromatic C-H and C=N stretching vibrations were observed at 3431, 2926, 2841

and 1628 cm^{-1}, respectively in the FTIR spectra of KSF-ILs. Cationic exchange of IL with clay is obscured by the wide bands of H-O-H and Si-O. Since the spectra of all KSF-ILs are similar, only the spectra of KSF, KSF-APBI-BF_4 and KSF-APBI-Br are given in Figure 3.4.

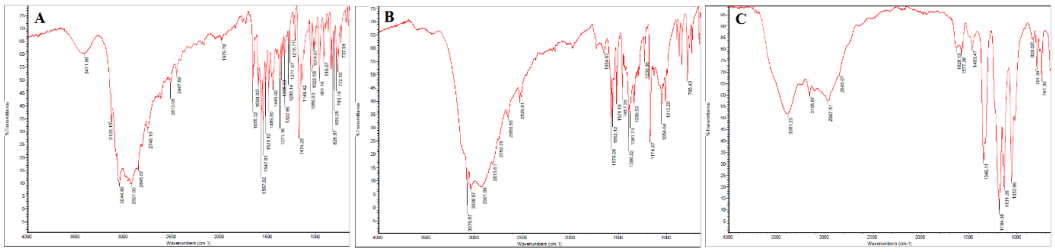


Figure 3.1 FTIR spectra of synthesized ionic liquids A) APMI-Br, B) APMI-BF_4, C) APMI-TFSI

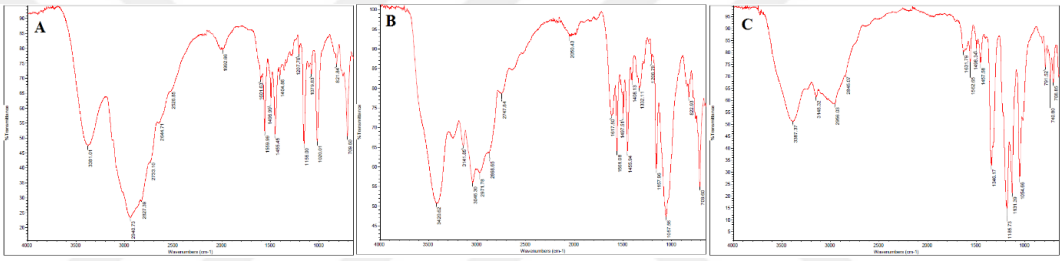


Figure 3.2 FTIR spectra of synthesized ionic liquids A) APBI-Br, B) APBI-BF_4, C) APBI-TFSI

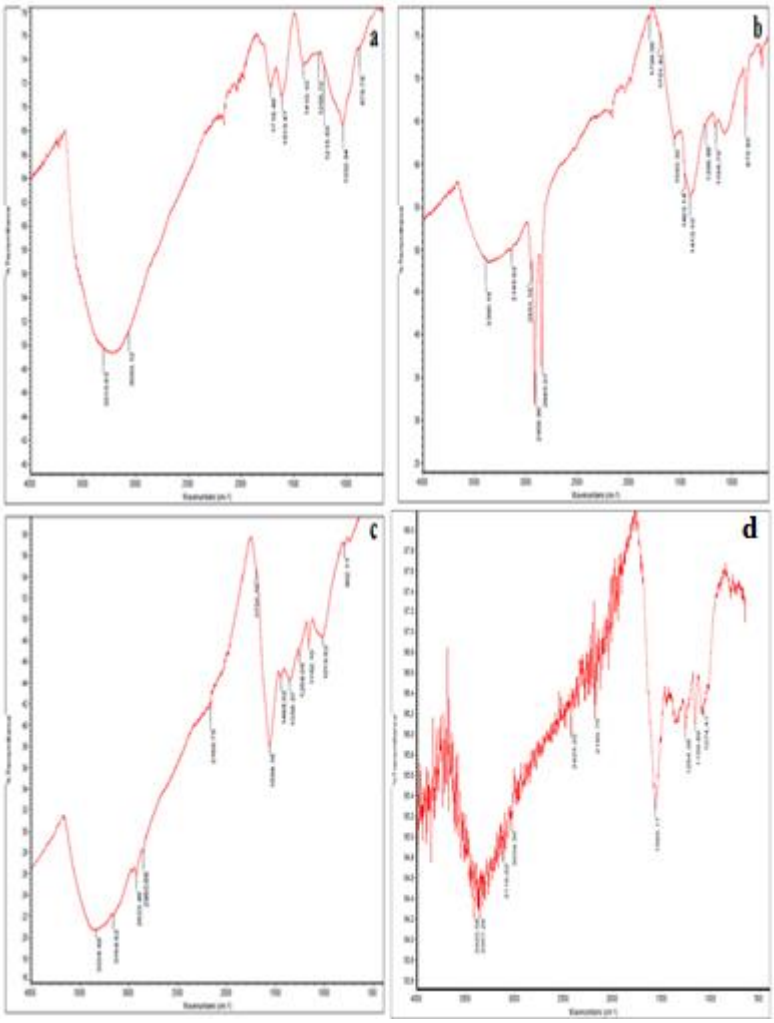


Figure 3.3 The FTIR spectra of a)GO, b) GO-APMI-BF_4, c) GO-APMI-TFSI d) GO-APMI-Br

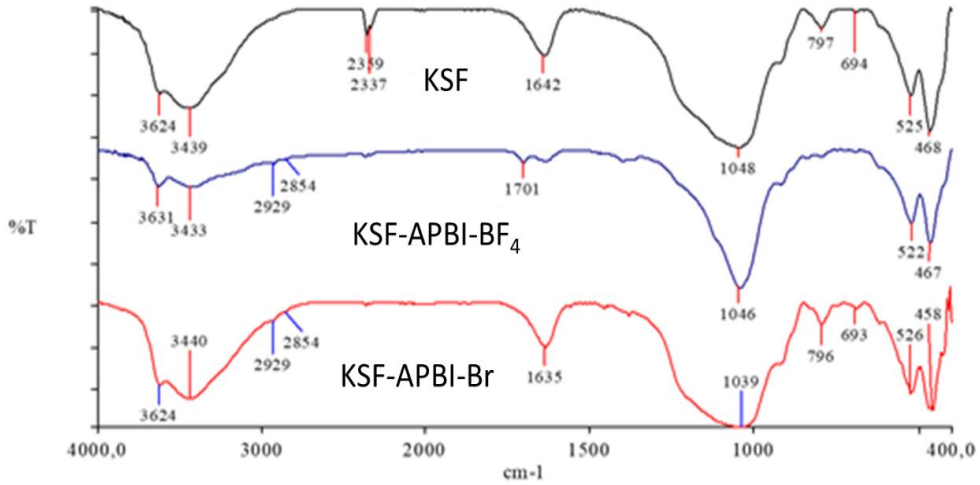


Figure 3.4 The FTIR spectra of KSF, KSF-APBI-BF_4, and KSF-APBI-Br

3.1.2 XRD Analyses

XRD analyzes of GO, GO-IL, KSF and some KSF-IL materials were performed at 2θ s^{-1} speed in the range of 3°-15° or 10°-60° and the corresponding XRD powder patterns are shown in Figure 3.5 and Figure 3.6. Sharp diffraction peaks observed at 2θ = 6° and 2θ = 29° in the XRD powder pattern of GO were similar with the literature (Stobinski et al., 2014). The appearance of new peaks at 2θ = 23.67°, 38.17° and 40.51° in XRD powder patterns explained the removal of water molecules and oxide groups and the interaction of GO with ILs.

The reflection peak in KSF at 2θ = 6.98° corresponds to 1.27 nm (Altınışık, Seki, & Yurdakoc, 2009). KSF-ILs showed peaks at 5.92-5.71°, which corresponds to basal spaces of approximately 1.50-1.55 nm. After the interaction of ILs and KSF, the reflection peaks shifted less than 1.27° and the basal gap shifted less than 0.28 nm. In Bragg's law, the shift of diffraction peak to a smaller angle is because of the rise in the d-space which the cation exchange reaction takes place (Pei, Xing, Xia, Wang, & Luo, 2019; Yalçınkaya, Pelit, Guney, & Turkmen, 2014; Takahashi, Shirai, & Fuji, 2012). In addition, the interaction between KSF and ILs was shown by the diffraction peaks seen at 2θ = 9° and 12.8° in the prepared coating materials.

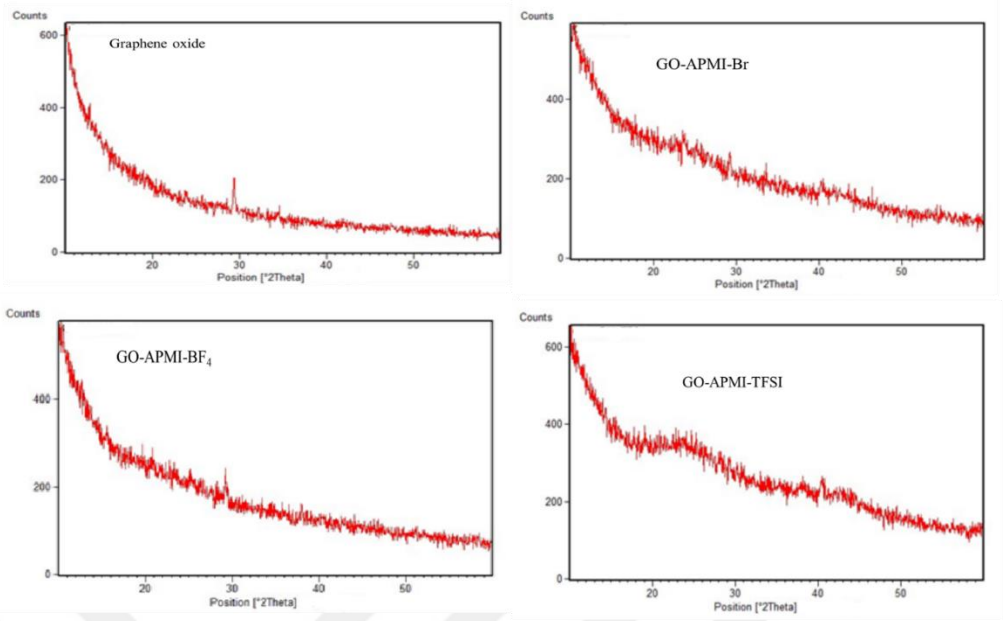


Figure 3.5 XRD powder patterns of GO, GO-APMI-Br, GAPMI-BF_4 and GO-APMI-TSFI

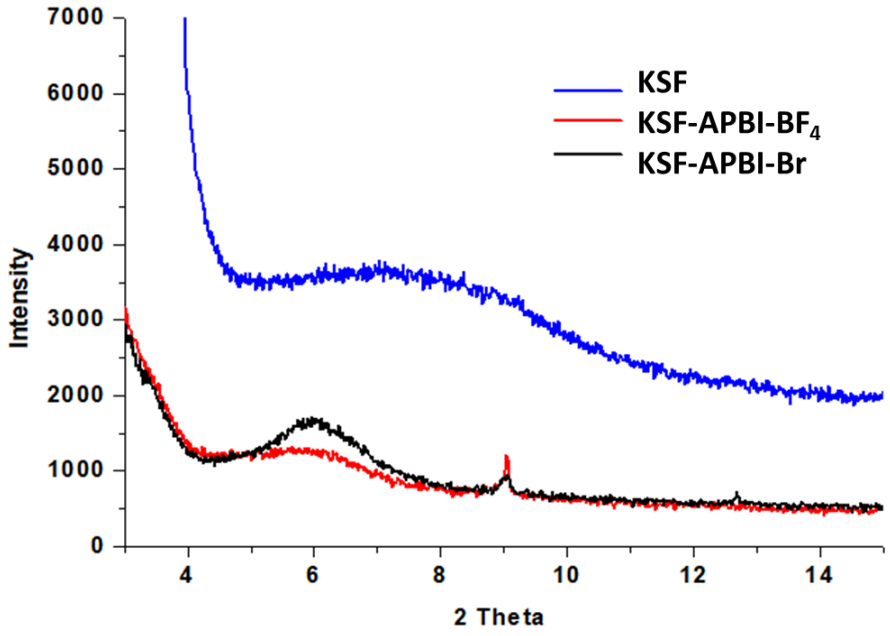


Figure 3.6 XRD powder patterns of KSF, KSF-APBI-BF_4 and KSF-APBI-Br

3.1.3 TG Analyses

In the thermal analysis of synthesized ionic liquids and prepared GO-IL and KSF-IL materials, the sample was taken into a platinum container and analyzed in the range of 30- 450 °C or 30- 600 °C in N_2 flow. TG curves of some ILs synthesized and coating materials were given in Figure 3.7, 3.8. A mass loss of ~ 4-5% was observed in all ionic liquids at ~ 100 °C due to the removal of water or moisture in their structures. Thermal decomposition temperatures of ionic liquids were obtained in the range of 224- 330 °C. GO-IL materials decreased in mass due to ~ 3-8% water loss at around 80 °C. In the thermal gravimetry analysis curves (Figure 3.7), GO-IL materials with less thermally labile oxygen functional group lost mass with a low slope (95 to 70%) in the range of 200-450 °C. Modification of GO with ILs caused the increase in its thermal stability, and the degradation curves of GO-ILs are lower sloped, but the degradation curve of GO is sharp.

In the TG curve of KSF clay (Figure 3.8), the mass losses up to 100°C were due to the free water loss between the layers of the KSF. KSF-IL composites showed slightly better thermal stability than KSF, which can be explained by the chemical adsorption of small amounts of ionic liquid with KSF as indicated in the previous work (Zhou et al., 2009; Ding, Wang, Zhan, & Wang, 2006).

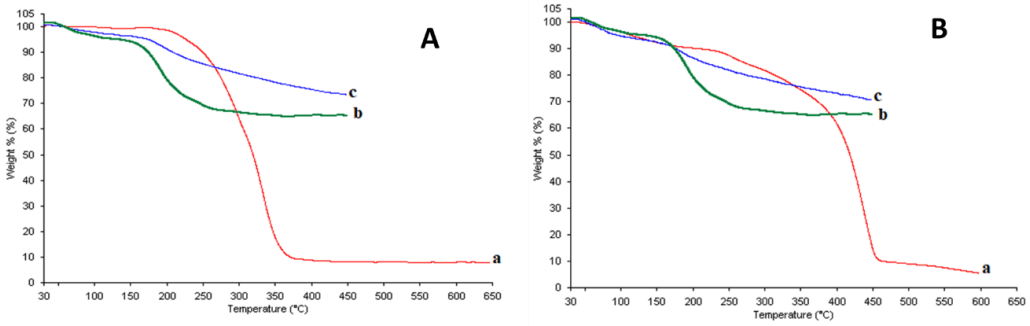


Figure 3.7 TG curves of APMI-BF_4 (a), GO (b), GO-APMI-BF_4 (c) in left side (A), and APMI-TFSI (a), GO (b), GO-APMI-TFSI (c) in right side (B)

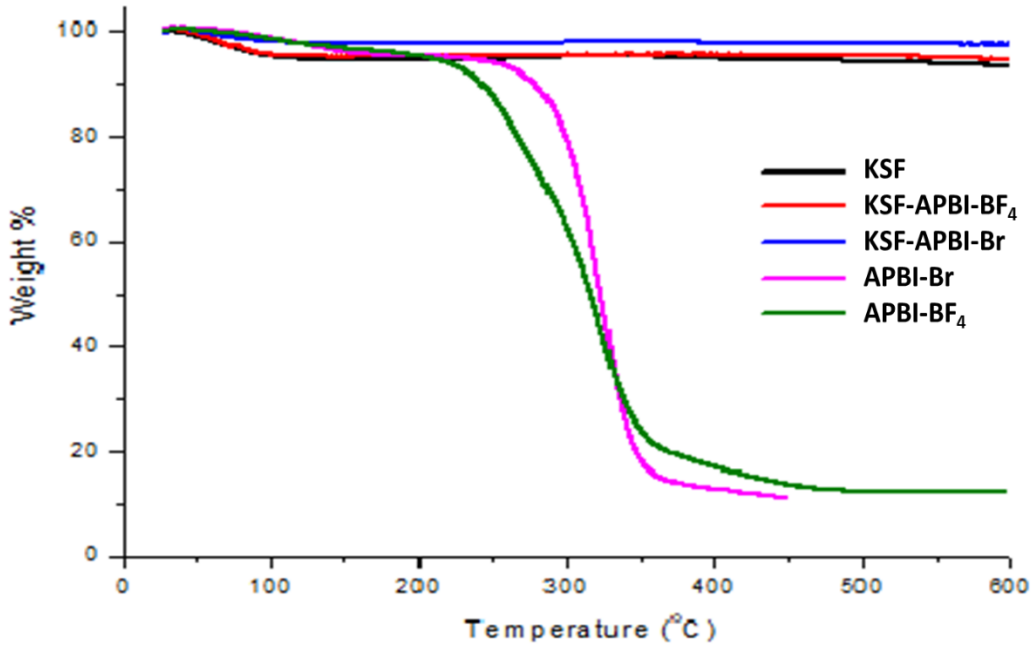


Figure 3.8 TG curves of KSF, KSF-APBI-BF_4, KSF-APBI-Br, APBI-Br and APBI-BF_4

3.1.4 SEM-EDS Analyses

SEM images, EDS graphs of GO, some GO-IL materials and GO-IL coated fibers were shown in Figure 3.9 and Figure 3.10. In the SEM images of the GO, the GO layers are seen as flat surfaces with wrinkled edges. It was observed that the surfaces of the materials were rougher after interacting with the ILs, indicating that the ILs were mounted on the GO plates. The fact that GO-IL's have a much rougher and more porous surface indicates that the coating materials have a large surface area. The surface of the coated fiber shows that a homogeneous and porous coating was provided. The porous structure of the coating indicates that the surface area and extraction capability of the fiber increases. The thickness of GO-IL fibers was in the range of 20-27.5 μm. As illustrated in Figure 3.9, C and O were main elements of GO, and S, N, F, B and Br, extra elements. The presence of these elements on the surface of fiber verified the ILs coated on GO.

In Figure 3.11, SEM images of KSF-IL and KSF coated fiber surfaces were shown. The surface morphology and the composition of KSF-IL fibers were performed by SEM and EDS, respectively. The surface of the fiber was clearly shown in Figure 3.11,

and a thickness of coating was calculated as 15-18 μm. The peaks of Cr, C and Ni belong to the wire base. As observed in Figure 3.11, the elemental composition of the coating materials was detected by EDS (from IL- S, C, N, Br, B and F and from KSF - Al, Si, O, Mg, Fe, and K). This has been shown to successfully coat KSF-APBI-Br and KSF-APBI-BF_4 on stainless steel wire.

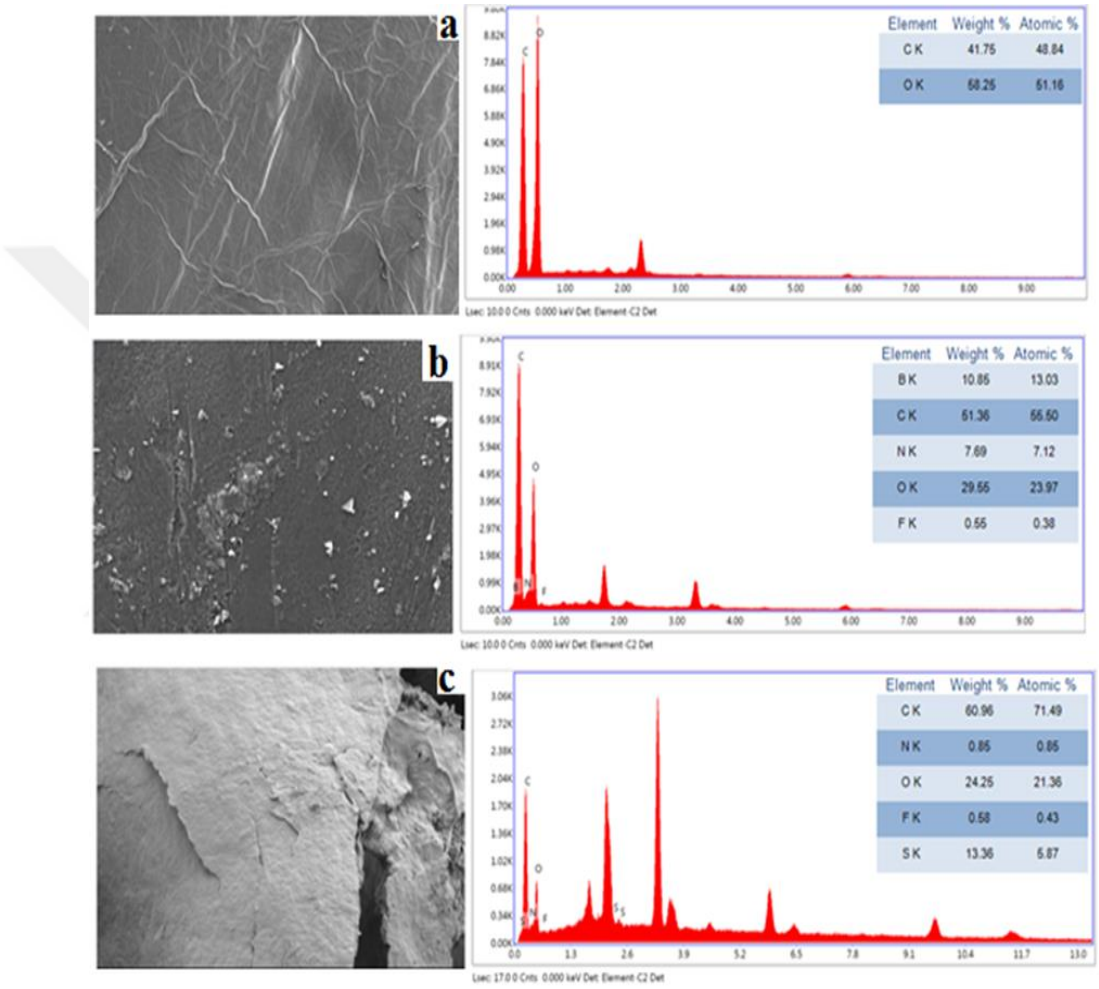


Figure 3.9 The SEM/ EDS analysis of a) GO, b) GO-APMI-BF_4, c) GO-APMI-TFSI

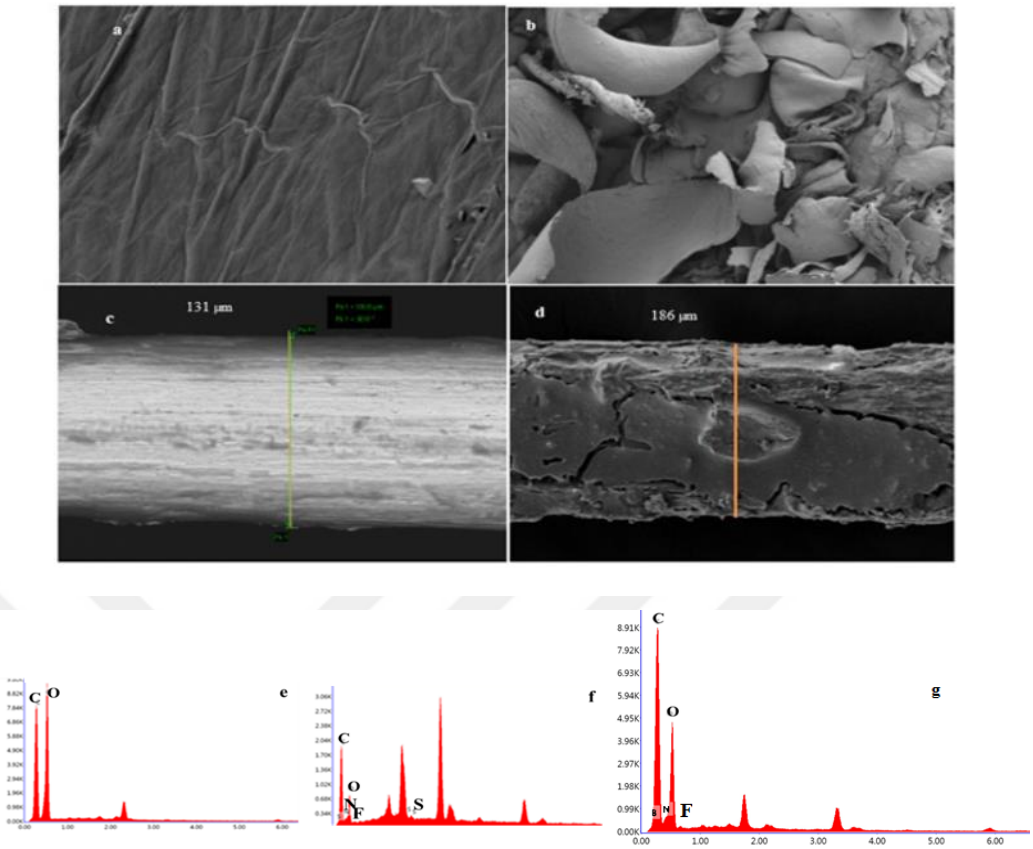


Figure 3.10 SEM and EDS images of GO, 2 µm, 10000 X (a); GO-APMI-TSFI, 20 µm, 500 X (b); etched steel wire, 20 µm, 1000 X (c); GO-APMI-TSFI coated fiber, 20 µm, 1000 X (d); EDS graph of GO (e); EDS graph of GO-APMI-TFSI (f), EDS graph of GO-APMI-BF_4 (g)

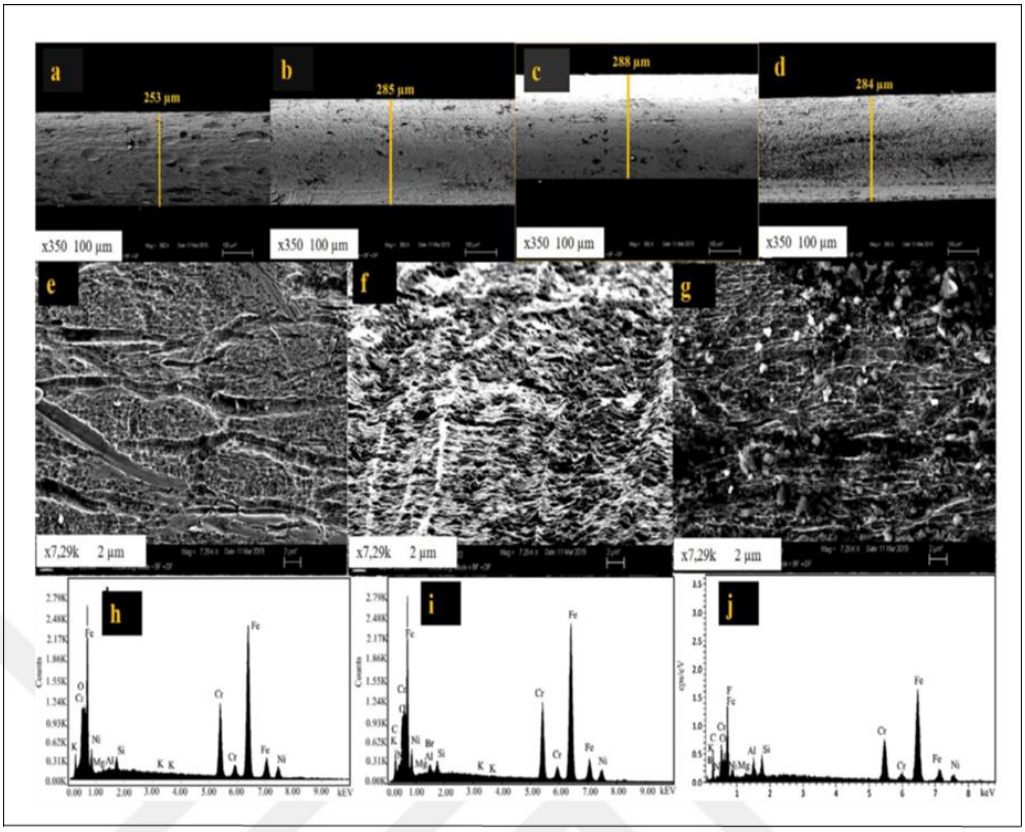


Figure 3.11 SEM images of etched steel wire (a), KSF (b), KSF-APBI-Br (c) and KSF-APBI-BF_4 (d) coated fibers; SEM images of fiber surfaces of KSF (e), KSF-APBI-Br (f), and KSF-APBI-BF_4 (g); EDS graphs of KSF (h), KSF-APBI-Br (i), KSF-APBI-BF_4 coated fibers (j)

3.2 Optimization of Desorption Process in the Determination of Phenolic Compounds by Chromatographic Methods

3.2.1 GC-MS

Phenolic compounds need to be derivatized before their analysis by GC-MS. Therefore, the derivatization method needs the optimization of some parameters such as selection of proper derivatization reagent, reaction volume and derivatization temperature and derivatization time in GC-MS analysis. The derivatizing reagents (Figure 3.12) used in this study are ETMS, BSTFA and TMCS. Trimethylsilyl (TMS) derivatives are less polar, volatile and have thermal stability. Due to these properties, they have been used in derivatization process, an active hydrogen in -OH, =NH, -NH, -COOH or -SH group in target molecules is taken out and the rest group bound to trimethyl silane group (Figure 3.13). SPME fibers used in immersion mode are dried

under nitrogen flow before derivatization process due to hydrolyzation of TMS reagents. Very few studies based on derivatization of polyphenolic compounds have been reported. Derivatization reagents can be directly added to sample solution. In SPME process, the fiber is exposed to derivatizing reagent for certain time at certain temperature. Another way can be adding to GC vial before desorption in GC injection port (Viñas, Campillo, Martínez-Castillo, & Hernández-Córdoba, 2009). In this study, the derivatization process was done on fiber after the extraction of the phenolic compounds. For this purpose, several variables were optimized. Trimethylsilane (TMS) reagents including BSTFA and TMCS are frequently used in the preparation of silylated derivatives of phenolic analytes. In most studies, the derivatization reagent of BSTFA: 1% TMCS has been used for the determination of phenolic compounds (Yuan et al., 2017; Bustamante, Cardenas, von Baer, Pastene, Duran-Sandvoval, Vergara, & Mardones, 2017).

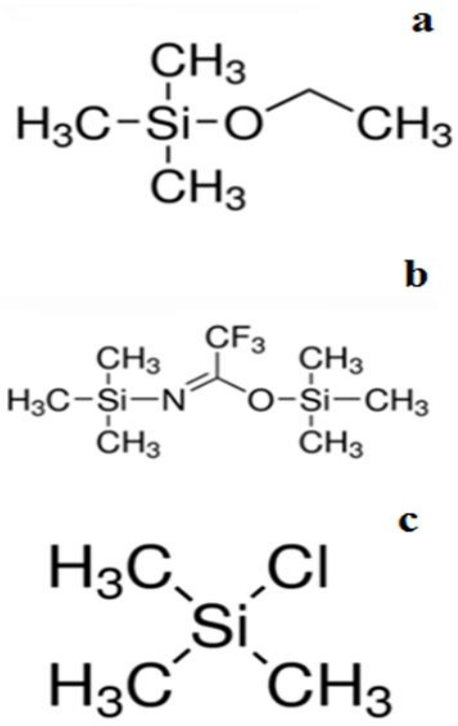


Figure 3.12 The structure of derivatization reagents a) ETMS, b) BSTFA, c) TMCS (Schummer et al., 2009)

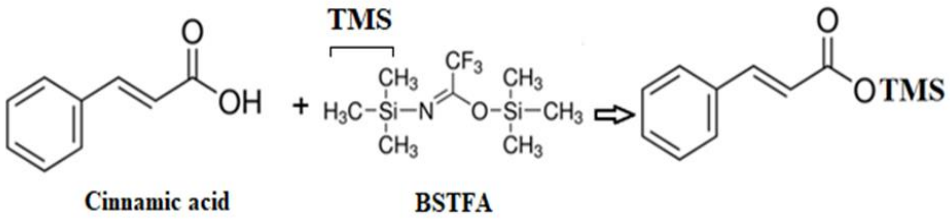


Figure 3.13 The derivatization reaction (Schummer et al., 2009)

In this study, phenolic compounds standards prepared in methanol were firstly derivatized with ETMS by modification of Proestos et al., method (Proestos, Sereli, & Kmaitis, 2004). For this purpose, several variables were investigated such as molar ratio (1:0.5, 1:1, 1:2) and derivatization temperature (70 and 75 °C) and derivatization time (45, 90, 180 min and 24 h). The optimum values with ETMS were determined as 1:1 molar raito, 75 °C and 24 h. Due to the weak reactivity and the long treatment time of ETMS, the derivatization reagent and the derivatization process were changed.

On-fiber derivatization, the fiber is exposed to derivatization reagent after or before extraction process. Firstly, the analytes were sorbed by the fiber and then the SPME fiber was subjected to the vapour of the derivatization reagent (Dietz, Sanz, & Cámara, 2006). In this study, secondly, BSTFA derivatization reagent was used for derivatization. Finally, the mixture of BSTFA: TMCS (9:1) that was previously used with successful results by Canini and co-workers (Cainini, Alesiani, D'Arcangelo, & Tagliatesta, 2007) was used (Figure 3.14). Additionally, derivatization temperature, derivatization time and volume of derivatization reagent were optimized. On the other hand, the SPME fiber that applied to SPME method must be dried before derivatization in nitrogen atmosphere. So, the drying time was also optimized. With GO-IL coated fibers, the optimum values are summarized: derivatization reagent BSTFA: TMCS (9: 1), drying time 5 min, derivatization temperature 60 °C, derivatization time 15 min and derivatization volume is 20 μL.

The derivatization temperature (30-50-60-75 °C), derivatization time (15-30-45-90 min) and reagent volume (10-20-30-50 μL) were also optimized using KSF-IL coated

fibers. Derivatization was carried out by usage of BSTFA: TMCS (9: 1) reagents (conditions: 20 µL, 60 °C, 15 min) as done with GO-IL coated fibers (Fig. 3.15).

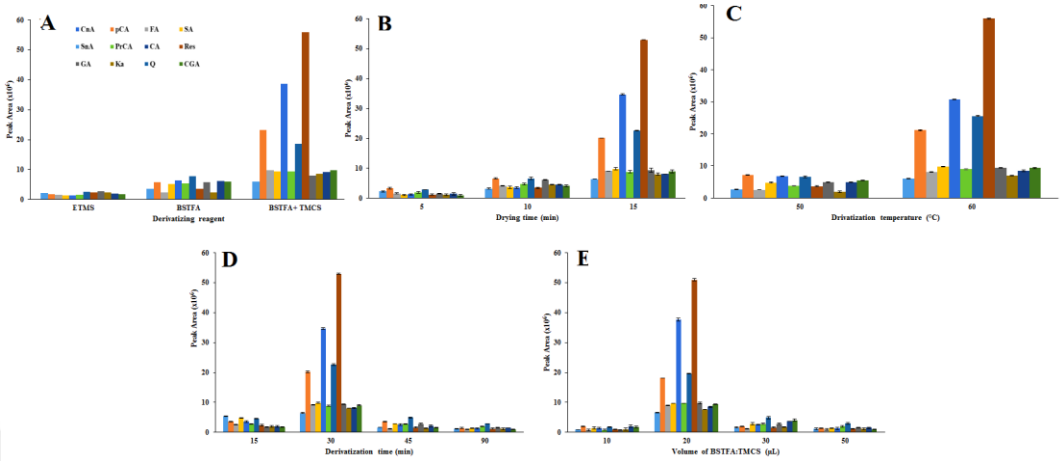


Figure 3.14 Optimization of derivatization parameters. A) derivatization reagent, B) drying time, C) derivatization temperature, D) derivatization time, E) derivatization reagent volume. Fiber GO-APMI-TFSI

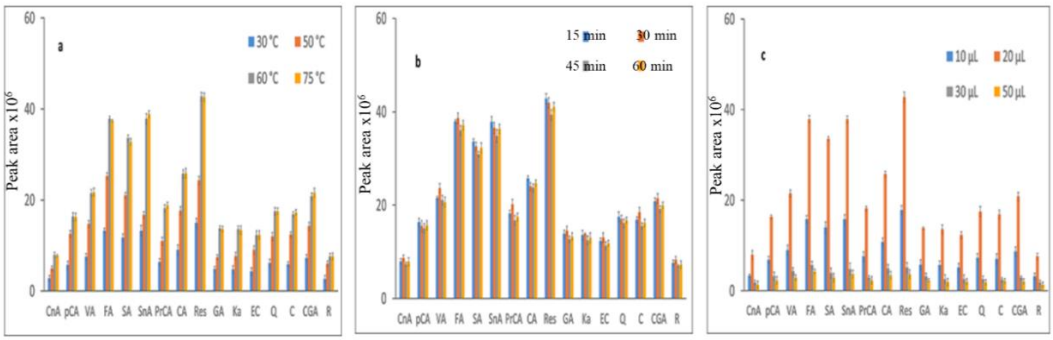


Figure 3.15 Optimization of derivatization parameters. a) derivatization temperature, b) derivatization time, c) derivatization reagent volume. Fiber KSF-APBI-BF_4

Desorption temperature and desorption time for the desorption by GC-MS can have importance in the extraction of analytes from the surface of fiber. The desorption temperature was investigated at 175 °C and 200 °C for GO-APMI-BF_4 coated fiber and 200 °C and 250 °C for GO-APMI-TFSI coated fiber and the effect of desorption time on the extraction efficiency as 2 and 5 minutes (Figure 3.16). The optimum desorption temperature was determined as 175 °C for -BF_4 and 250 °C for -TFSI ended fibers. The desorption time was optimized as 5 min for both fibers. Also, the optimization of desorption conditions (temperature and time) were examined with

KSF-IL fibers. Because of the similar results obtained in all prepared KSF based SPME fibers, the extraction performances taken with KSF-APBI-BF_4 fiber were shown in Figure 3.17. Desorption was reached maxima at 200 °C for 5 minutes. During the experiments, fiber was put into another injection port at the desorption temperature for an additional 3 minutes after desorption process to prevent the accumulation of analytes on the surface of fibers.

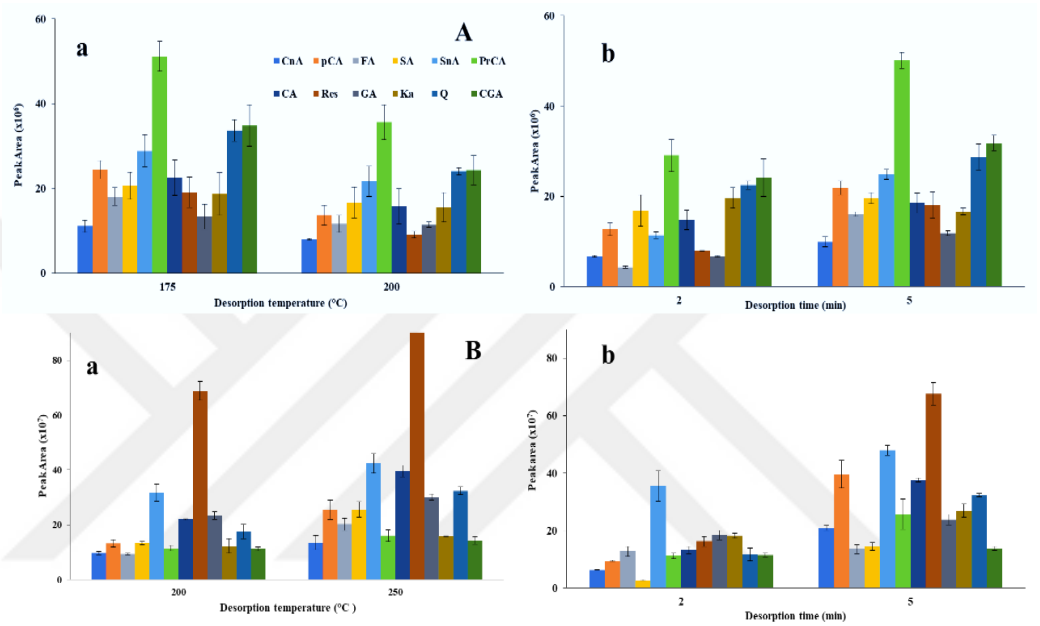


Figure 3.16 Desorption optimization in SPME-GC-MS method. A) with GO-APMI-BF_4 and B) with GO-APMI-TFSI, a) desorption temperature, b) desorption time

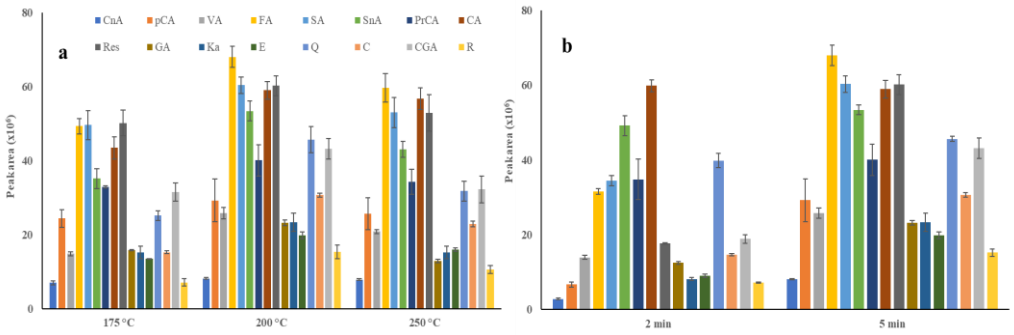


Figure 3.17 Desorption optimization in SPME-GC-MS method. a) desorption temperature, b) desorption time. Fiber: KSF-APBI-BF_4

3.2.2 LC

In LC method, desorption process parameters such as elution solvent type, elution temperature and elution time were studied. To get best desorption of analytes from the fiber, methanol, acetonitrile and MeOH: ACN: 2% HAc were selected as elution solvent types. The best elution was succeeded with MeOH: ACN: 2% Hac which was used as mobile phase for target molecules (Figure 3.18). Also, the extraction efficiency of analytes were controlled by applying two different elution temperature such as 30 °C and 40 °C and elution time for 2.5-5-10-15 min. Based on the results obtained in Figure 3.18, elution of target molecules was achieved using ACN: 2% HAc for 10 minutes at 30 °C.

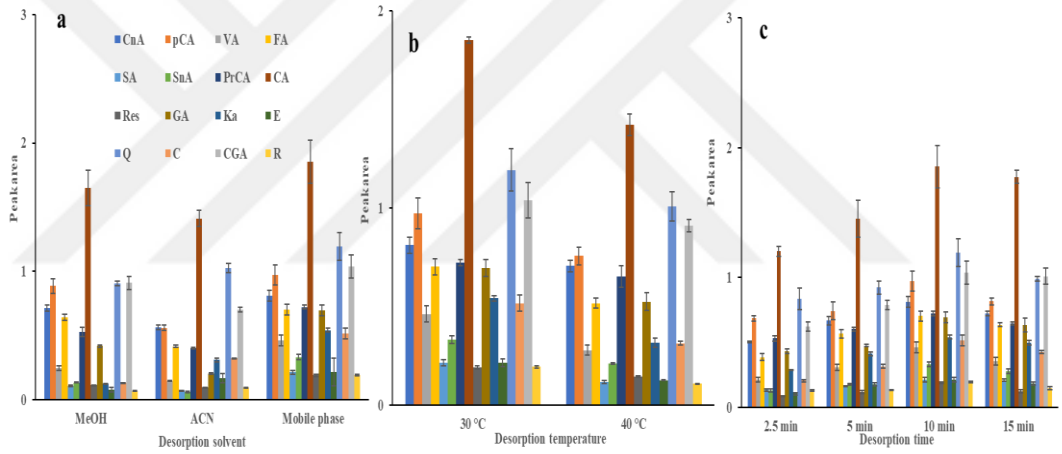


Figure 3.18 Desorption optimization in SPME-LC/DAD method. a) desorption solvent type, b) desorption temperature, and c) desorption time. Fiber: KSF-APBI-BF_4

3.3 Study of SPME Coupled to GC-MS Using GO-IL Fibers for Wine Analysis

To obtain the optimal extraction conditions, some parameters were investigated. The pH of sample solution, extraction time, extraction temperature, and the salt effect were examined. All experiments were performed in triplicate with the test solutions spiked with each analyte at 100 μg L^{-1}.

3.3.1 Optimization of SPME Method

The pH of the test solution affects the retention of analytes on the surface of the coating material of the SPME fiber. Depending on the polarities of the analyte and the fiber surface, interactions take place between them. The pH of the sample solution was adjusted to obtain reproducible extraction efficiency, considering the pK_a of the analytes. Depending on the pK_a values of phenolic compounds, the effect of pH was investigated at pH 4 and 8 using GO-APMI-BF_4 and GO-APMI-TFSI coated fibers. It was observed that the extraction efficiencies of all phenolic compounds examined, except for syringic acid and gallic acid with GO-APMI-TFSI coated fiber, were higher at pH 8 (Figure 3.19 a and Figure 3.20 a). Since phenolic compounds are in anionic form at pH 8, it can be stated that electrostatic interactions are effective in the adsorption between ionic analytes and imidazolium cation (Pei, & Huang, 2016; Yang et al., 2019). In addition, it is thought that the adsorption occurs between the aromatic rings, -OH, -OCH_3, and -COOH groups in the structure of phenolic compounds and the surfaces of GO-IL fibers cause by dipole-dipole and π-π interactions. Since the extraction was more effective overall at pH 8, further studies were continued at pH 8.

In HS-SPME and uncommonly in SPME methods, increasing the ionic strength of the aqueous solution positively affects the adsorption of the analytes to the fiber surface and the solubility of the analytes in aqueous solution by improving the peak areas. Therefore, the effect of ionic strength for uptake of phenolic compounds was investigated at three different NaCl concentrations (0%, 10%, and 25% (w/v)). As seen in Figure 3.19 b and Figure 3.20 b, the addition of NaCl at 10% caused initially a slight increase caused a slight increase in the peak areas of phenolic compounds and subsequently a decrease in the extraction efficiency. The presence of high salts can increase the viscosity of the solution, this causes reduction in the mass transfer from sample solution to surface of fiber. Therefore, 10% NaCl solution was used in subsequent experiments.

Extraction temperature is another important factor for extraction efficiency. It affects the mass transfer and extraction time in the SPME method. Therefore, the

extraction temperature affects the extraction efficiency of analytes. So, the extraction temperature effect was studied at 30 °C and 70 °C by applying extraction time as 30 min. When the temperature profiles obtained in Figure 3.19 c and Figure 3.20 c were examined, it was determined that the highest extraction performances were obtained using GO-IL fibers at 30 °C. The peak abundance of target compounds was declined with the increase in temperature above 30 °C. This can be explained by the fact that high temperature causes rapid movement of neutral or ionized phenolic compounds but decreases their diffusion.

The extraction time affects the interaction of the analyte solution and the fiber surface. Therefore, the extraction time was investigated between 15 and 90 minutes, at an optimum extraction temperature of 30 °C. As seen in Figure 3.19 d and Figure 3.20 d, the extraction time reached its optimum value of 30 minutes at 30 °C.

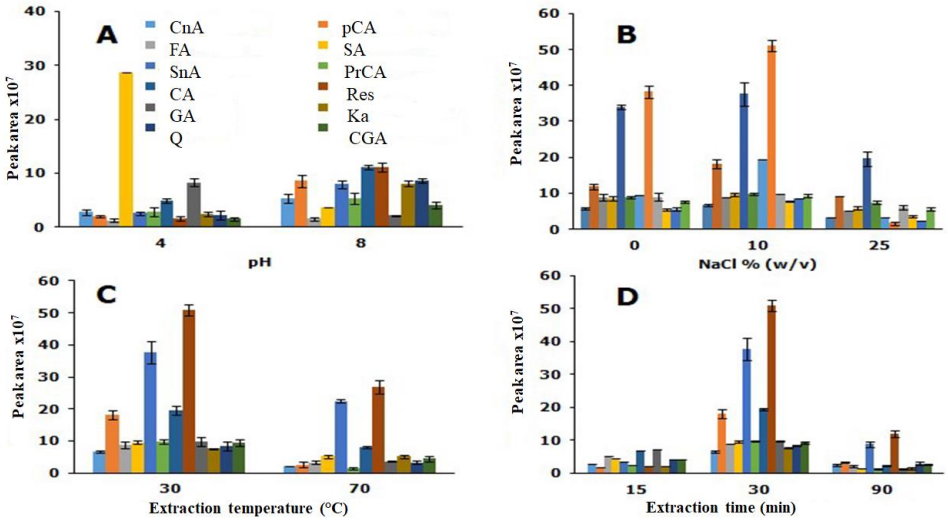


Figure 3.19 Optimization of SPME-GC-MS method with GO-APMI-TFSI fiber, A) pH, B) salt effect, C) extraction temperature, and D) extraction time

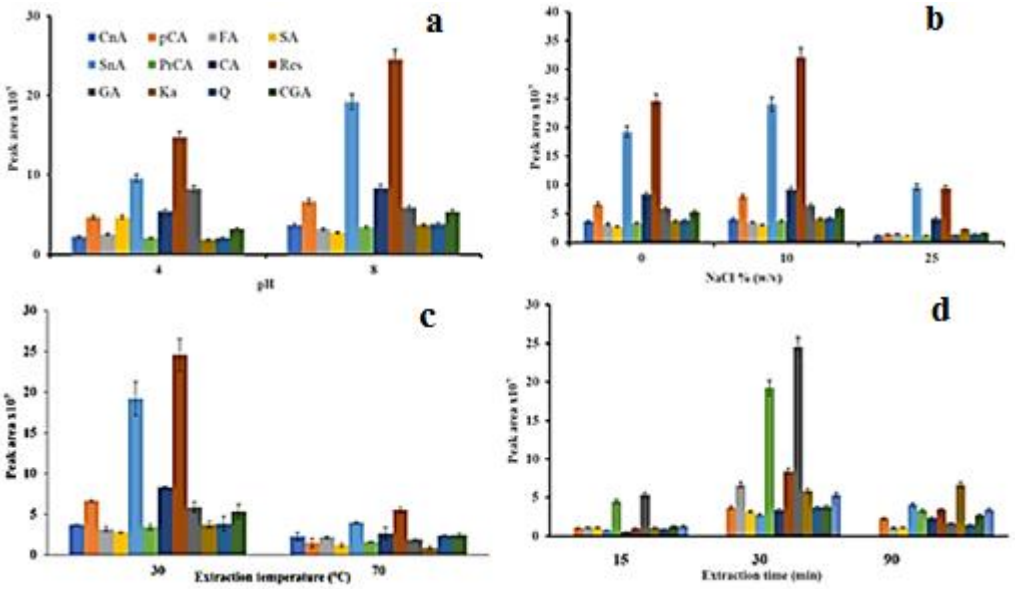


Figure 3.20 Optimization of SPME-GC-MS method with GO-APMI-BF_4 fiber a) pH, b) salt effect, c) extraction temperature, and d) extraction time

3.3.2 Comparison of Extraction Performances of GO-IL Coated Fibers with Only GO Coated Fiber and Commercial Fibers

In the SPME method, optimization study was performed with commercial fibers to compare the extraction efficiency of GO-APMI-BF_4 and GO-APMI-TSFI coated fibers with PA, DVB/CAR/PDMS, CAR/PDMS and CW/PEG commercial SPME fibers. Prior to the extraction experiments, in accordance with the manufacturer's recommendation, PA fiber at 280 °C, DVB/CAR/PDMS fiber at 270 °C, CAR/PDMS fiber at 300 °C and CW/PEG fiber at 240 °C were conditioned for 30 minutes in the injection port of GC. SPME extraction experiments with commercial fibers were performed at pH 8 and 400 rpm at a concentration of 100 µg L^{-1} phenolic compound in the range of 15-60 min to obtain the optimum extraction time. The highest extraction efficiency was obtained for all commercial SPME fibers at 30 minutes. The extraction temperature was investigated at 30 °C and 70 °C at the optimized extraction time. Experimental results showed that the optimum extraction temperature for all commercial fibers was 30 °C. Although the optimal extraction parameters of the commercial fibers were the same as the GO-IL coated fibers, the extraction efficiency of the prepared fibers was much higher than the commercial fibers studied (Figure 3.21). Among commercial SPME fibers, the peak areas of CnA and Ka with PA fiber

and CnA peak area with CW/PEG fiber were observed higher than the prepared GO-IL fibers. As seen in Figure 3.21, the presence of IL caused a very high increase in the extraction efficiency of phenolic compounds compared to GO coated fiber alone.

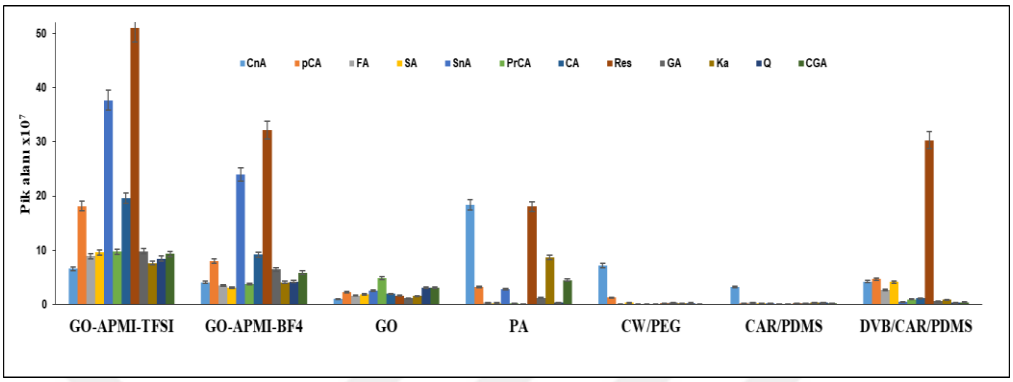


Figure 3.21 Comparison of extraction efficiencies of GO-IL coated fibers with GO and commercial fibers

Since the presence of IL causes dipole-dipole, π-π and electrostatic interactions, it provides high adhesion on the studied fiber surface (Pei, & Huang, 2016; Yang et al., 2019). While it was expected that the activity of polar phenolic compounds with the hydrophilic BF_4^- anion was higher, slightly higher efficiency with the hydrophobic $TFSI^-$ anion has been obtained. At the pH determined in this interaction, it can be explained by the effect of the synergy created by GO and ILs, as well as the intermolecular interactions mentioned above. Since the extraction efficiency of the GO-APMI-TFSI fiber from the two prepared GO-IL fibers was slightly higher, only the analytical performance of the specified fiber and real sample analyses were performed under optimum conditions. In addition, analytical performance and real sample analyses were not performed due to the very weak extraction efficiency of commercial fibers.

3.3.3 Method Validation

Analytical performances of GO-APMI-TFSI and GO-APMI-BF_4 coated fibers, linearity, coefficient of determination (r^2), detection limit (LOD), repeatability of single fiber and inter-fiber were investigated for the quantification of 12 phenolic compounds by the proposed. The calibration curve was studied with ten calibration

points in three parallels. The results obtained are given in Table 3.1 and Table 3.2. The linear range was determined in the range of of 0.25-1000 μg L^{-1} for pCA, FA, CA and GA and 0.1-1000 μg L^{-1} for other phenolic compounds with the coefficient of determination of 0.997-0.999 using GO-APMI-TFSI fiber. The limits of detection (S/N = 3) of the studied phenolic compounds are between 0.02-0.1 μg L^{-1}. The reproducibility of the method for a single fiber was examined in three parallel repetitions for each concentration of 10 points from which the calibration line was established for each phenolic compound. Relative standard deviations (RSD) for single fiber ranged from 0.71% to 14.82%. With three separate fibers prepared with the same coating process, fiber-to-fiber reproducibility ranged from 1.64 to 5.87% at 100 μg L^{-1} concentration.

The linear range was between 0.5 and 500 μg L^{-1} for the phenolic compounds studied except Q and CGA using GO-APMI-BF_4 fiber with the coefficient of determination of 0.997-0.999. The LOD values were ranged as 0.05 μg L^{-1} for SnA, PrCA, Q and CGA and 0.1 μg L^{-1} for others. The precision was ranged from 1.80 to 14.74% and the fiber-to-fiber reproducibility was ranged from 3.36 to 6.40% at 100 μg L^{-1} concentration. The linear range was wider and LODs were lower with GO-APMI-TFSI fiber.

Table 3.1 Validation parameters of SPME-GC method using GO-APMI-TFSI fiber for phenolic compounds

PCs	Linear range (µg/L)	R^2	LOD (µg/L)	Precision (RSD, %)	Fiber-to-Fiber (RSD, %)
CnA	0.1-1000	0.998	0.05	2.48-11.37	2.39
pCA	0.25-1000	0.998	0.1	2.22-9.23	2.79
Fa	0.25-1000	0.998	0.1	2.57-9.68	2.18
SA	0.1-1000	0.999	0.02	1.32-14.82	4.68
SnA	0.1-1000	0.997	0.02	0.71-13.29	3.04
PrCA	0.1-1000	0.997	0.02	0.92-8.42	5.71
CA	0.25-1000	0.997	0.05	3.48-14.74	1.64
Res	0.1-1000	0.997	0.02	0.93-10.53	4.79
GA	0.25-1000	0.997	0.02	0.90-9.85	2.33
Ka	0.1-1000	0.997	0.02	0.78-9.85	5.87
Q	0.1-1000	0.998	0.02	3.14-9.24	4.06
CGA	0.1-1000	0.997	0.02	2.73-11.92	2.25

Table 3.2 Validation parameters of SPME-GC method using GO-APMI- BF_4 fiber for phenolic compounds

PCs	Linear range (µg/L)	R^2	LOD (µg/L)	Precision (RSD, %)	Fiber-to-Fiber (RSD, %)
CnA	0.5-500	0.997	0.1	2.11-9.38	3.98
pCA	0.5-500	0.998	0.1	2.34-8.11	3.65
FA	0.5-500	0.998	0.1	2.58-10.52	4.47
SA	0.5-500	0.997	0.1	1.66-9.22	5.52
SnA	0.5-500	0.999	0.05	3.80-14.08	4.32
PrCA	0.5-500	0.999	0.05	2.79-8.77	5.24
CA	0.5-500	0.999	0.1	1.61-13.90	3.36
Res	0.5-500	0.998	0.1	3.23-14.74	4.84
GA	0.5-500	0.998	0.1	3.51-8.09	4.54
Ka	0.5-500	0.998	0.1	1.80-5.65	5.24
Q	0.25-500	0.998	0.05	2.07-8.52	5.65
CGA	0.25-500	0.998	0.05	3.93-6.24	6.40

3.3.4 Wine Analysis by SPME Coupled to GC-MS Method

Using the lab-made GO-IL coated fiber, analyses of 12 phenolic compounds were performed in 2 red wine, 2 white wine and four fruit wine samples by SPME-GC-MS method (Table 3.3). With the equilibrium-based and non-exact SPME method, the free analytes are extracted from the samples, the quantitative results represent the free analyte concentration in the samples (Aresta, Cotugno, Massari, & Zambonin, 2018). The recoveries were investigated for the accuracy of the method by adding 12 phenolic compounds to the wine samples at 5 and 200 μg L^{-1} concentrations.

SnA was not detected in all studied wine samples. FA was not found in one of the red wine samples, all other phenolic compounds were detected. Except for Ka, Q and Res, other phenolic compounds were detected in white wine samples. No additional CnA was found in fruit wines. Ka and Res in black mulberry wine, FA and SA in blueberry wine, Ka, Q and Res in melon wine and SA, PrCA, GA and Res in red plum wine were not detected. To evaluate the accuracy of the SPME-GC-MS method based on the prepared GO-APMI-TFSI fiber, the recovery of phenolic compounds was 75.4-99.8% for red wines and 75.2-99.9% for white wines, according to the results of the analysis with wine samples. It was obtained with BSS values of less than 13.7% in the range of 72.8-99.9% for fruit wines. Typical GC chromatograms obtained from GC-MS analyses of fruit wines, white wine and red wine samples developed after SPME, and red wine GC chromatogram with added phenolic compound mixture standard were given in Figure 3.22.

Table 3.3 Analysis results of phenolic compounds in wine samples using GO-APMI-TFSI fiber (n= 3)

Wine samples	Added ($\mu g\ L^{-1}$)		CnA	pCA	FA	SnA	CA	CGA	SA	PrCA	GA	Ka	Q	Res
Red 1	0	Found[a] ($\mu g\ L^{-1}$)	52.9± 4.2	3.6±0.3	ND	ND	1.1± 0.4	98.3± 1.3	440.0± 9.5	1.8± 0.1	464.0± 7.3	133.4± 7.0	88.8± 8.9	93,5± 1,9
	5	Recovery,RSD%[b]	96.3, 10.6	95.8, 7.0	84.6, 6.4	76.8, 8.9	82.4, 8.2	99.2, 10.6	99.7, 3.8	88.4, 11.6	99.7, 3.3	99.8, 10.2	99.7, 2.3	99.0, 3.3
	200	Recovery,RSD%	94.6, 3.4	96.2, 6.2	88.5, 4.0	77.0, 4.8	89.5, 1.6	93.9, 4.0	95.3, 2.7	91.0, 10.0	97.5, 2.9	97.6, 4.4	96.7, 4.3	93.0, 2.3
Red 2	0	Found[a] ($\mu g\ L^{-1}$)	58.3± 7.1	255.7± 0.3	382.0± 0.3	ND	2.8± 0.2	108.0± 0.6	270.0± 0.6	1.4± 0.8	194.0± 3.2	233.0± 12.3	103.7± 0.9	81.5± 0.6
	5	Recovery,RSD%[b]	96.7, 6.13	97.6, 9.7	97.5, 8.4	75.4, 7.2	84.0, 8,9	98.9, 2.4	99.7, 3.9	91.6, 7.1	99.8, 6.3	99.5, 10.6	99.2, 6.8	98.9, 0.2
	200	Recovery,RSD%	95.2, 5.2	96.8, 7.2	96.4, 3.3	79.2, 4.9	87.5, 1.5	89.9, 2.5	96.8, 1.0	92.5, 6.6	90.1, 5.6	98.1, 6.2	94.3, 5.4	97.6, 1.0
White 1	0	Found[a] ($\mu g\ L^{-1}$)	2.9± 0.7	69.6± 0.9	85.1± 0.4	ND	308.0± 1.4	7.8± 2.7	54.8± 0.2	496.0± 0.1	491.0± 4,9	ND	ND	ND
	5	Recovery,RSD%[b]	89.4, 5.3	95.1, 8.3	99.0, 12.4	75.2, 2.8	99.6, 1.8	86.0, 9.4	98.1, 6.6	99.9, 4.0	99.9, 7.8	83.5, 10.1	80.5, 8.3	80.0, 4.9
	200	Recovery,RSD%	91.8, 2.5	93.1, 5.8	91.9, 1.5	78.4, 2.4	97.4, 3.8	92.9, 6.5	93.4, 1.3	97.3, 0.9	97.3, 4.0	88.9, 8.1	88.7, 5.8	84.7, 0.6
White 2	0	Found[a] ($\mu g\ L^{-1}$)	ND	166.5± 0.5	280.0± 0.1	ND	133.5± 0,1	7.9± 0.3	94.5± 0.4	205.0± 0.4	362.5± 2.2	ND	ND	ND
	5	Recovery,RSD%[b]	83.0, 4.8	97.1, 10.7	99.5, 3.8	78.2, 7.8	99.0, 2.6	82.2, 8.2	98.2, 3.5	99.7, 4.2	99.7, 2.6	80.0, 5.3	80.8, 9.0	74.2, 11.0
	200	Recovery,RSD%	88.6, 1.1	95.1, 8.1	92.3, 0.7	77.0, 2.7	95.2, 4.1	85.8, 0.3	85.4, 0.7	94.9, 0.3	95.4, 0.8	88.3, 6.2	83.6, 5.2	83.3, 1.6
Black Mulberry	0	Found[a] ($\mu g\ L^{-1}$)	ND	238.0± 0.1	301.0± 0.2	ND	415.5± 2.7	447.5± 0.1	280± 0.5	1.6± 0.4	407.0± 0.8	ND	338.0± 1.8	ND
	5	Recovery,RSD%[b]	86.4, 5.0	99.4, 6.8	99.6, 3.1	76.6, 9.2	96.5, 5.6	99.6, 5.7	99.5, 6.6	81.6, 5.4	99.6, 2.4	78.2, 4.4	99.5, 1.1	86,2, 10,2
	200	Recovery,RSD%	92.6, 0.8	93.6, 0.2	93.8, 3.5	80.9, 0.9	93.3, 3.6	95.1, 1.2	93.4, 0.3	83.7, 3.2	92.9, 2.9	89.9, 2.1	94.7, 0.3	88.8, 5.4
Blueberry	0	Found[a] ($\mu g\ L^{-1}$)	ND	107.0± 0.8	ND	ND	415.5± 4.5	3.7± 0.5	ND	1.7± 0.2	416.0± 2.1	448.5± 7.9	469.0± 6.7	482.0± 0.2
	5	Recovery,RSD%[b]	82.2, 8.3	88.7, 8.3	80.7, 8.1	77.6, 3.1	99.5, 5.2	82.2, 6.3	80.0, 10.5	79.6, 8.4	99.6, 8.2	99.6, 6.9	99.7, 7.8	99.7, 4.5
	200	Recovery,RSD%	88.5, 4.6	86.1, 5.1	82.4, 4.1	85.1, 2.3	96.8, 6.5	91.0, 4.7	83.8, 4.3	83.2, 5.2	86.1, 0.9	86.2, 5.0	87.1, 1.9	83.0, 3.5
Melon	0	Found[a] ($\mu g\ L^{-1}$)	ND	371.0± 1.8	311.0± 2.0	ND	453.0± 0.1	294.0± 0.2	272.0± 4.5	441.0± 7.9	312.0± 0.8	ND	ND	ND
	5	Recovery,RSD%[b]	81.8, 4.63	99.7, 1.3	99.8, 1.5	73.4, 7.7	99.8, 0.9	99.6, 1.8	99.6, 3.9	99.9, 0.6	99.8, 1.8	84.2, 4.8	84.4, 5.1	81.6, 1.4
	200	Recovery,RSD%	93.7, 1.3	95.7, 0.5	95.9, 0.8	89.9, 0.1	96.4, 0.7	95.1, 0.8	92.4, 0.7	96.0, 0.6	91.4, 0.9	93.5, 1.1	88.5, 1.2	96.5, 2.8

Table 3.3 continous

Red plum	0	Found ($\mu g\ L^{-1}$)	ND	448.0± 8.5	364.0± 4.2	ND	957.0± 6.6	314.0± 4.2	ND	ND	ND	398.0± 8,5	975.0± 10,6	ND
	5	Rocevery,RSD%	84.6, 4.8	99.7, 1.3	99.5, 6.0	75.2, 1.4	99.8, 6.8	99.4, 3.96	78.8, 8.4	72.8, 7.2	77.6, 4.1	99.5, 1.9	99.8, 9.2	76.2, 6.6
	200	Recovery,RSD%	91.0, 4.4	98.3, 1.4	93.1, 3.5	85.1, 4.4	95.1, 0.8	97.4, 3.0	89.5, 7.9	83.0, 3.2	81.7, 2.7	96.6, 2.2	97.0, 0.5	93.3, 3

[a]mean ± standard deviation; [b]%RR : Relative recovery percentage; ND: not detected

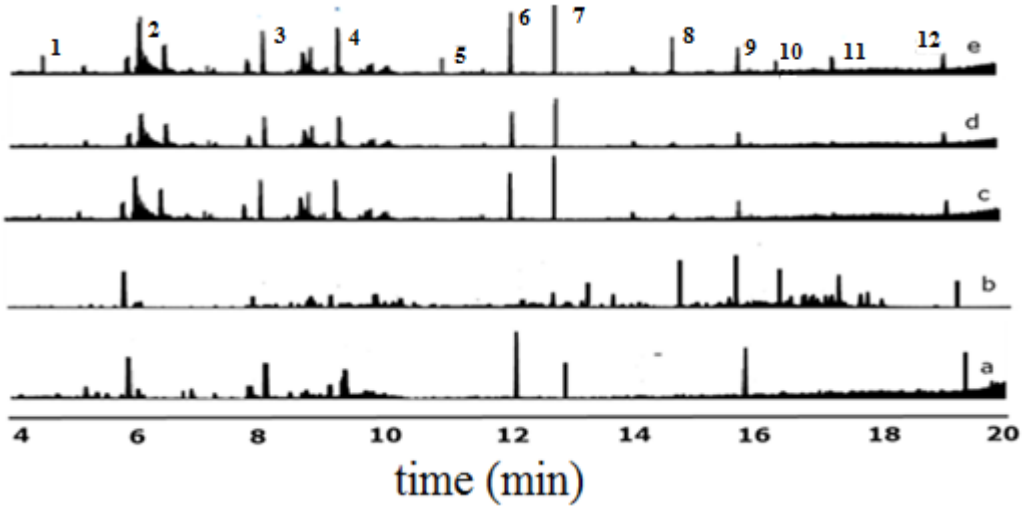


Figure 3.22 Total ion chromatograms of wine samples in SIM mode using SPME-GC-MS method and GO-APMI-TFSI fiber a) melon wine, b) blueberry wine, c) red wine, d) red wine with 12 phenolic compounds added at a concentration of 200 μg L^{-1}, e) standard solution of phenolic compounds. Peak identification: (1) CnA, (2) pCA, (3) FA, (4) SA, (5) SnA (6) PrCA, (7) CA, (8) Res, (9) GA, (10) Ka, (11) Q, (12) CGA

3.4 Study of SPME Coupled to GC-MS Using KSF-IL Fibers

To obtain the optimal extraction conditions, some parameters were investigated. The pH of sample solution, extraction time, extraction temperature, speed of stirring, and the salt effect were examined. All experiments were performed in triplicate with the test solutions spiked with each analyte at 100 μg L^{-1}.

3.4.1 Comparison of the Lab-made Fibers

In this study, KSF clay was modified with imidazolium cation with alkyl and aryl group having hydrophilic (Br^-, BF_4^-) and hydrophobic anions ($TFSI^-$) and used as SPME coatings. In Figure 3.23, the extraction performances of the prepared fibers were given. The extraction efficiency of fibers with IL with $TFSI^-$ anions was found to be lower than the others. Other coatings including Br^- and BF_4^- ions presented better extraction performances. In addition, better extraction performances were obtained with KSF coatings containing benzylimidazolium cation, which provides a higher degree of π-π and H-bond interactions. At the end of this, KSF-APBI-Br and KSF-APBI-BF_4 fibers were used for optimization studies.

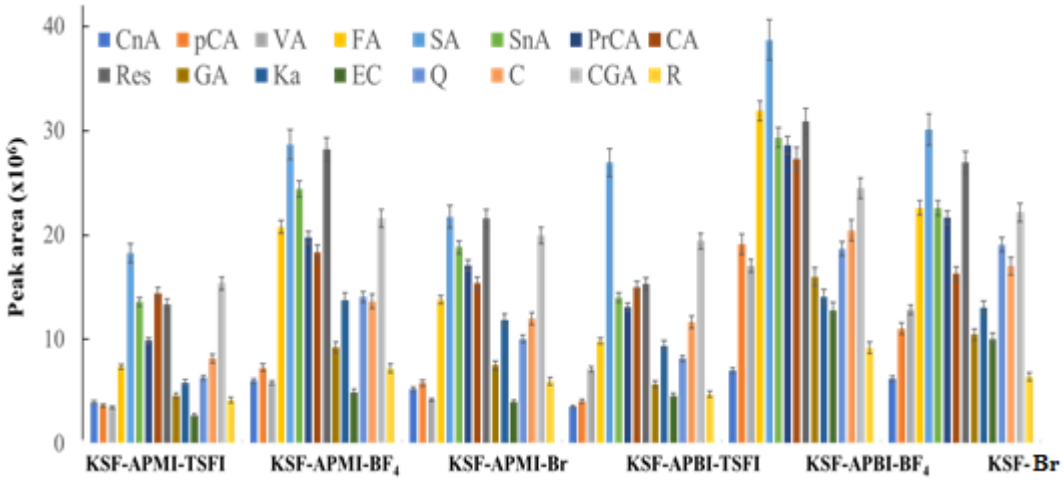


Figure 3.23 Extraction performances of KSF-IL fibers in SPME coupled to GC-MS

3.4.2 Optimization of SPME Method

The extraction performance of coatings was influenced from the applied temperature due to mass transfer rate of analytes from sample to fiber surface. The extraction efficiency of analytes was examined at 30, 50 and 70 °C using IL coated KSF-APBI-Br and KSF-APBI-BF_4 fibers with benzylimidazolium cation containing hydrophilic anion groups. As shown in Figure 3.24A and Figure 3.25A, the highest peak area was observed at 30 °C for the prepared fibers. Also, the extraction time was studied because of the dependence of SPME method to equilibrium. Therefore, SPME experiments were performed for 15-30-45-60 min. With respect to Figure 3.24B and Figure 3.25B, the highest extraction at 45 min was obtained for each analyte. The extraction performance was decreased after 45 min. The reason for this may be that the analytes retained on the fiber surface go back into solution due to some properties of the coating materials (Zhang, & Lee, 2011; Abdolhosseine, Ghiasvand, & Heidari, 2017). At longer extraction times, the coating surface could be in race with water molecules for analytes (Vinas, Campillo, Martinez-Castillo, & Hernandez-Cordova, 2009; Papageorgiou, Lambropoulou, Morrison, Namiesnik, & Plotka-Wasylka, 2018). Therefore, further studies were performed at 30 °C for 45 min.

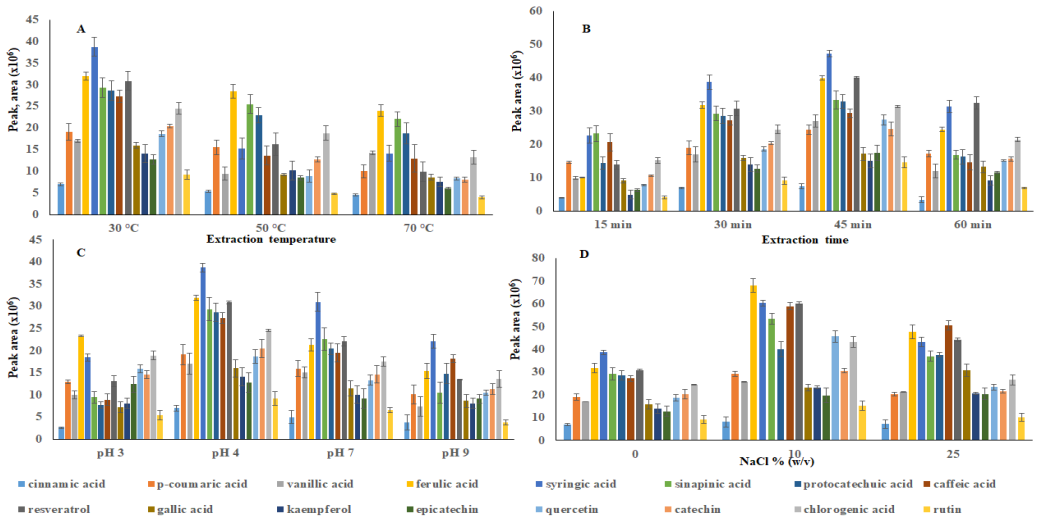


Figure 3.24 Optimization profiles of SPME-GC-MS method with KSF-APBI-BF_4 fiber A) extraction temperature, B) extraction time, C) pH, and D) salt effect

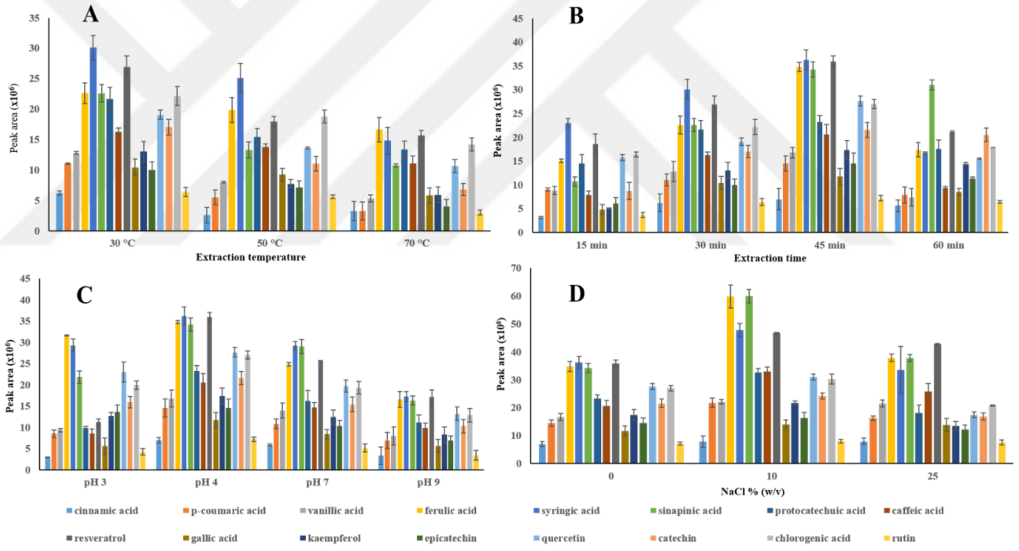


Figure 3.25 Optimization profiles of SPME-GC-MS method with KSF-APBI-Br fiber A) extraction temperature, B) extraction time, C) pH, and D) salt effect

The effect of pH on the sorption of phenolic compounds is based on their pKa values. Selected pH values were from 3 to 9. At pH4, the enhancement in signal reached the maxima and decreased at higher pHs (Figure 3.24C and Figure 3.25C). At pH4, most of the analytes exist in neutral or protonated and others in ionized form. This situation was explained by H-bonding and π-π interactions with imidazolium group in ionic liquid and analytes (Pei, & Huang, 2016; Yang et al., 2019). Additionally, electrostatic interaction and coordinative bonding are responsible in the

interaction of clay with analytes (Ahmat, Thiebault, & Guegan, 2019). When pH studied is above pKa values, analytes exist in anionic form. So, in these pHs, the weak interactions were seen, negative ions in the surface of clay and anions of analytes repelled each other.

The addition of salt can influence the solubility of organic compounds. Salting-out effect can increase the ionic strength and viscosity of working solution. In immersion mode of SPME, the extraction performance of analytes can be negatively affected. The NaCl concentration in the studied solutions was set to 0% -10%-25% (w/v). The studied phenolic compounds were extracted more at 10% salt and then declined at higher salt concentrations (Figure 3.24D and Figure 3.25D).

The rate of stirring generally can affect the transfer of analyte mass to the fiber surface. Therefore, the sample solutions were stirred at different rates, ranging from 200 to 800 rpm. The highest extraction efficiencies were obtained at 400 rpm (Figure 3.26). As seen from the results, the optimum conditions of the SPME method based on KSF-IL were determined as pH 4, an extraction time of 45 minutes at 30 °C at 400 rpm in 10% NaCl environment.

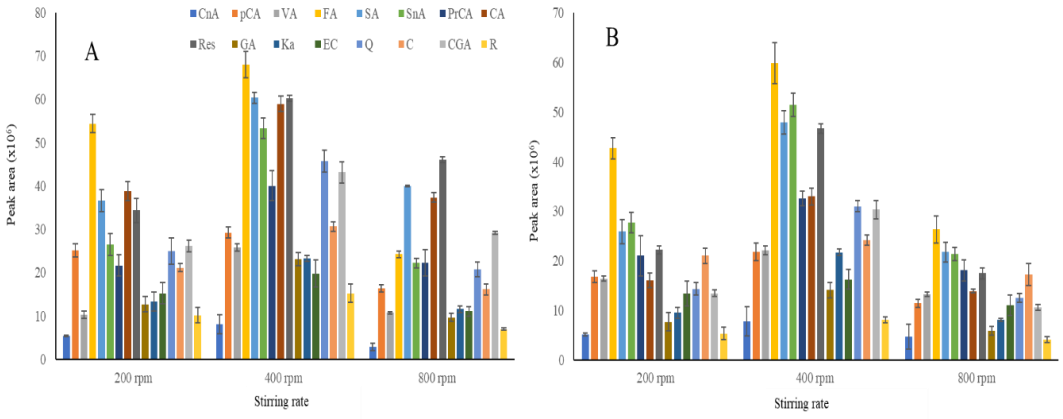


Figure 3.26 Stirring effect in the optimization of the SPME-GC-MS method A) KSF-APBI-BF_4 fiber, B) KSF-APBI-Br fiber

3.4.3 Comparison of Extraction Performances of KSF-IL Coated Fibers with Only KSF Coated Fiber and Commercial Fibers

To present the better extraction performance of ionic based clay coatings, KSF-APBI-Br and KSF-APBI-BF_4 coated fibers with high extraction efficiency, phenolic compounds were extracted by SPME coupled to GC and SPME coupled to LC methods using KSF and commercial fibers under optimum conditions. As seen in Figure 3.27, KSF-APBI-Br and KSF-APBI-BF_4 coated fibers have better and higher extraction performances for target compounds than KSF. The reason can be the existence of ionic liquids in the clay composites. With the addition of IL to the KSF, the interlayer spaces of the clay increased with the introduction of ILs between the layers of the clay (Figure 3.6), indicating the presence of ionic liquid on the KSF between the layers. This may cause the clay to allow ionic liquid to be present on the fiber, to add strength to the fiber, and to prevent/slow down the flow of material over the fiber, allowing reusability. In addition, the extraction performances of the lab-made fibers were compared with 4 different commercial fibers which are suitable for semi-polar and polar compounds. As observed in Figure 3.27, DVB/CAR/PDMS and PA commercial fiber in SPME coupled to GC and CAR/PDMS and CW/PEG commercial fibers in SPME coupled to LC presented better extraction performance than other commercial fibers. The extraction power of lab-made fibers (KSF-APBI-Br and KSF-APBI-BF_4) was highest within the the studied commercial fibers.

Analytical performances were performed under optimum conditions with KSF-APBI-Br and KSF-APBI-BF_4 fibers, which showed higher extraction performance than the other prepared KSF-IL prepared fibers. In addition, analytical performance and real sample analyses were not performed with commercial fibers due to their very weak extraction efficiency for phenolic compounds.

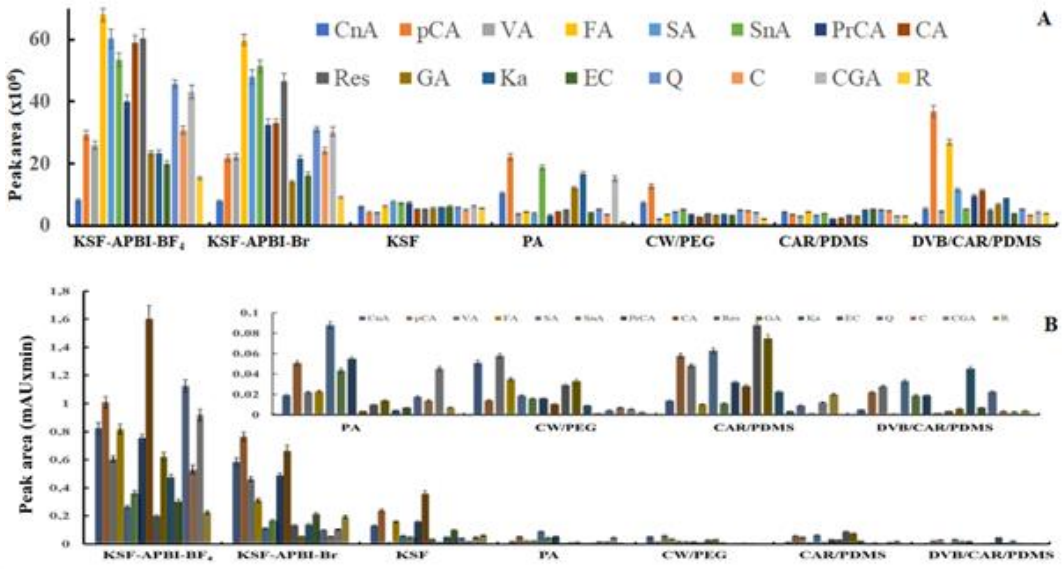


Figure 3.27 Comparison of extraction performances of KSF-IL coated fibers with KSF and commercial fibers. A) SPME coupled to GC-MS method; B) SPME coupled to LC method

3.4.4 Method Validation

The quality parameters of the methods such as linearity, detection limit (LOD), repeatability, and reproducibility were determined under optimized conditions. The linearity of the methods was studied by preparing the analytical curve for each analyte with seven points. The studied concentration range was from 0.01 to 1000 μg L^{-1}. The linear calibration curves were obtained with the coefficient of determination of r^2 > 0.993 in GC and >0.994 in LC (Table 3.4 and Table 3.5). Linear ranges of the lab-made fibers were larger in SPME combined to GC-MS method than SPME combined to LC method LOD (S/N = 3) values obtained by LC method were higher than that of in GC method.

Considering the repeatability study, relative standard deviations (RSDs) using single fiber was ranged from 0.9 to 4.2% for 100 μg L^{-1} in the GC method and ranged from 1.0 to 4.2% 250 μg L^{-1} in the LC method with five parallel runs. Inter-fiber reproducibility of the lab-made fibers in the GC method were little better than in the LC method.

The fiber lifetime was evaluated by taking into account the signals obtained after the SPME method using the lab-made fibers. The peak areas of the target analytes were not changed significantly with the prepared fibers after 150 extraction and desorption cycles. This shows that the lab-made fibers have high durability and thermal.

Tablo 3.4 Validation parameters of SPME coupled to GC method using KSF-IL fibers

	Linear range ($\mu g\ L^{-1}$)	R^2	LOD ($\mu g\ L^{-1}$)	RSD (%) One fiber (n = 3)	Fiber-to-Fiber* (n= 3)	Linear range ($\mu g\ L^{-1}$)	R^2	LOD ($\mu g\ L^{-1}$)	RSD (%) One fiber (n = 3)	Fiber-to-Fiber (n= 3)
	KSF-APBI-BF_4					KSF-APBI-Br				
CnA	0.02-1000	0.998	0.01	2.57-9.47	2.63	0.1-1000	0.999	0.05	2.80-11.78	3.13
pCA	0.02-1000	0.998	0.01	1.87-8.29	2.72	0.1-1000	0.998	0.05	2.44-9.39	3.87
VA	0.02-1000	0.994	0.01	4.62-7.76	2.13	0.1-1000	0.995	0.05	5.11-8.01	3.65
FA	0.02-1000	0.997	0. 1	1.96-9.28	2.51	0.1-1000	0.999	0.05	3.04-9.91	2.94
SA	0.02-1000	0.999	0.01	2.86-6.85	2.97	0.1-1000	0.999	0.05	2.99-10.39	3.96
SnA	0.02-1000	0.998	0.01	0.18-9.74	1.33	0.1-1000	0.999	0.05	2.06-5.84	2.60
PrCA	0.02-1000	0.999	0.01	1.04-9.69	2.67	0.1-1000	0.999	0.05	1.03-8.18	2.77
CA	0.02-1000	0.999	0.01	0.74-6.32	2.41	0.1-1000	0.999	0.05	2.68-9.19	2.87
Res	0.02-1000	0.998	0.01	2.45-8.18	2.87	0.1-1000	0.997	0.05	2.02-9.94	3.13
GA	0.02-1000	0.999	0.01	1.47-6.17	2.01	0.1-1000	0.999	0.05	2.24-4.08	2.84
Ka	0.02-1000	0.998	0.01	1.15-10.29	3.17	0.1-1000	0.999	0.05	1.57-9.90	3.84
EC	0.02-1000	0.995	0.01	2.38-7.27	4.43	0.1-1000	0.995	0.05	3.08-9.21	4.73
Q	0.02-1000	0.997	0.01	2.55-10.75	3.21	0.1-1000	0.999	0.05	2.51-9.92	4.15
C	0.02-1000	0.993	0.01	2.05-8.48	4.99	0.1-1000	0.994	0.05	2.15-8.26	5.94
CGA	0.02-1000	0.999	0.01	0.43-10.00	3.67	0.1-1000	0.999	0.05	0.50-7.04	3.82
R	0.02-1000	0.995	0.01	1.69-6.89	3.29	0.1-1000	0.995	0.05	1.71-7.00	4.84

***** 100 $\mu g\ L^{-1}$

Tablo 3.5 Validation parameters of SPME coupled to LC method using KSF-IL fibers

	Linear range ($\mu g\ L^{-1}$)	R^2	LOD ($\mu g\ L^{-1}$)	RSD (%) One fiber (n = 3)	Fiber-to-Fiber* (n= 3)	Linear range ($\mu g\ L^{-1}$)	R^2	LOD ($\mu g\ L^{-1}$)	RSD (%) One fiber (n = 3)	Fiber-to-Fiber (n= 3)	Linear range ($\mu g\ L^{-1}$)	R^2
	KSF-APBI-BF_4						KSF-APBI-Br					
CnA	0.5-1000	0.996	2.5	2.61-7.31	3.47	0.5-1000	0.994	2.5	3.11-8.54	3.21	0.5-1000	0.996
pCA	0.5-1000	0.996	2.5	0.21-4.97	4.54	0.5-1000	0.999	2.5	0.44-5.42	3.69	0.5-1000	0.996
VA	0.5-1000	0.997	2.5	1.39-5.14	3.55	0.5-1000	0.996	2.5	2.11-6.00	3.76	0.5-1000	0.997
FA	0.5-1000	0.997	2.5	0.62-6.73	2.89	0.5-1000	0.996	2.5	1.06-7.74	3.39	0.5-1000	0.997
SA	0.5-1000	0.995	2.5	0.87-3.34	4.66	0.5-1000	0.996	2.5	1.99-4.28	4.21	0.5-1000	0.995
SnA	0.5-1000	0.997	2.5	2.38-10.87	2.89	0.5-1000	0.994	2.5	2.26-9.85	2.95	0.5-1000	0.997
PrCA	0.5-1000	0.994	2.5	2.32-6.79	2.49	0.5-1000	0.995	2.5	3.12-8.81	2.80	0.5-1000	0.994
CA	0.5-1000	0.994	2.5	0.98-5.78	3.24	0.5-1000	0.994	2.5	1.58-6.91	4.33	0.5-1000	0.994
Res	0.5-1000	0.997	2.5	1.20-9.29	3.98	0.5-1000	0.994	2.5	3.01-10.49	5.60	0.5-1000	0.997
GA	0.5-1000	0.997	2.5	3.84-10.59	3.49	0.5-1000	0.999	2.5	2.54-8.14	3.44	0.5-1000	0.997
Ka	0.5-1000	0.997	2.5	2.08-9.85	4.97	0.5-1000	0.997	2.5	3.54-9.19	3.34	0.5-1000	0.997
EC	0.5-1000	0.999	2.5	1.00-3.02	5.88	0.5-1000	0.998	2.5	2.00-2.91	5.04	0.5-1000	0.999
Q	0.5-1000	0.996	2.5	3.88-6.58	3.30	0.5-1000	0.996	2.5	4.15-8.29	5.81	0.5-1000	0.996
C	0.5-1000	0.999	2.5	0.33-4.15	4.93	0.5-1000	0.997	2.5	1.08-5.05	4.43	0.5-1000	0.999
CGA	0.5-1000	0.998	2.5	3.75-6.57	3.84	0.5-1000	0.996	2.5	4.05-7.40	3.23	0.5-1000	0.998
R	0.5-1000	0.996	2.5	1.63-5.82	5.98	0.5-1000	0.996	2.5	1.81-6.08	4.71	0.5-1000	0.996

* 250 $\mu g\ L^{-1}$

3.4.5 Fruit Juice Analysis by SPME-GC-MS Method

Fruit juices were analyzed by both two chromatographic methods using KSF-APBI-BF4 fiber. Fruit juices were analyzed in two ways: freshly squeezed and kept in the refrigerator for 3 days. The level of analytes found in fruit juice samples was given in Table 3.6. Rutin was only determined in quince, orange, and starking apple juice. By SPME coupled GC method, at least 8 types of analytes were determined in the fruit juice samples. However, by the SPME coupled LC method, the number of detected analytes were lower since the lower sensitivity of the LC method. Analysis of the studied fruit juices by direct SPME obtained by both methods and GC and LC chromatograms with the standard phenolic compound mixture is given in Figure 3.28 and Figure 3.29 for the orange juice sample. As seen in the chromatograms, there was no interference in the retention times of the analytes. The extracted analyte is generally at the free form in the SPME method, but, in the solid phase extraction, the analyte exists in free or bound forms that can be analyzed (Mirnaghi, Mousavi, Rocha, & Pawliszyn, 2013). Therefore, the values of phenolic compounds obtained were like the other SPME methods given (Pei, & Huang, 2016; Chen, & Huang, 2017) but lower than the comprehensive methods (Agcam, Akyıldız, & Akdemir Evrendilek, 2014; Guine, & Barroca, 2014; Ljevar, et al, 2016). To show the accuracy of the proposed methods, juice samples were spiked with analyte as 200 μg L^{-1} and the results are given in Table 3.7 and Table 3.8. Recovery of analytes in fresh juice samples was in the range of 85 – 102 % with precision less than 4.6% RSD by GC analysis and 82 - 99% with precision less than 6.9% RSD by LC analysis. According to the obtained results, ionic liquid-based clay coatings presented good coating performances to extract polar analytes like phenolic compounds. As demonstrated in Table 3.6, the concentrations of analytes in the fruit juice samples were found similar to both two methods. Additionally, the comparison of GC and LC methods was done using a t-test (95% confidence level). No significant difference between GC and LC methods was statistically determined.

Tablo 3.6 Determination of analytes in fresh fruit juices by the proposed methods (n=3)

			Orange	Quince	Grapefruit	Mandarin	Pear	Pomegranate	Fuji apple	Starking apple	Golden apple
		Found (ug L^{-1})	$\bar{x}$±s (ug L^{-1})	$\bar{x}$±s (ug L^{-1})	$\bar{x}$±s (ug L^{-1})	$\bar{x}$±s (ug L^{-1})	$\bar{x}$±s (ug L^{-1})	$\bar{x}$ (ug L^{-1})	$\bar{x}$ (ug L^{-1})	$\bar{x}$ (ug L^{-1})	$\bar{x}$ (ug L^{-1})
CnA	GC	fresh	25.3±1.2	2.35±0.30	nd	nd	23.5±1.4	4.46±0.10	nd	5.30±1.26	2.84±0.40
		refrigerator	2.58±0.66	1.95±0.38	nd	nd	23.2±1.6	1.65±0.14	nd	7.54±1.00	2.52±0.05
	LC	fresh	28.9±0.8	nd	nd	nd	21.2±1.2	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	20.7±0.9	nd	nd	nd	nd
pCA	GC	fresh	36.4±0.9	2.25±0.10	2.21±0.36	39.3±2.4	1.59±0.18	1.44±0.02	1.49±0.13	1.57±0.13	1.43±0.61
		refrigerator	19.7±1.8	1.8±0.3	1.83±0.37	16.9±2.5	1.56±0.13	0.62±0.02	2.11±0.06	1.83±0.11	1.49±0.03
	LC	fresh	33.9±1.3	nd	nd	36.3±3.4	nd	nd	nd	nd	nd
		refrigerator	14.0±1.8	nd	nd	12.1±1.8	nd	nd	nd	nd	nd
VA	GC	fresh	97.3±0.3	16.7±2.1	112.3±1.3	36.6±4.2	352.5±5.1	1.21±0.10	7.83±0.83	9.18±0.82	6.93±0.28
		refrigerator	54.1±2.4	12.3±1.3	83.5±1.3	18.3±0.9	343.8±4.0	nd	7.08±0.11	9.00±0.18	6.25±0.18
	LC	fresh	95.9±0.7	13.5±3.2	110.8±2.5	31.7±1.7	302.7±3.8	nd	7.64±0.64	9.40±0.18	6.26±0.33
		refrigerator	51.3±1.3	12.3±2.7	81.4±1.2	17.2±1.1	331.7±1.8	ns	6.76±0.23	nd	6.25±0.80
FA	GC	fresh	27.6±3.7	nd	nd	31.9±1.5	0.87±0.06	0.74±0.06	0.80±0.02	0.80±0.03	0.80±0.02
		refrigerator	4.13±0.78	nd	nd	21.2±1.1	0.84±0.01	0.48±0.11	0.97±0.08	0.89±0.15	0.76±0.06
	LC	fresh	31.1±0.9	nd	nd	30.1±0.9	nd	nd	nd	nd	nd
		refrigerator	7.33±0.62	nd	nd	9.18±0.96	nd	nd	nd	nd	nd
SA	GC	fresh	11.8±1.1	nd	1.05±0.18	nd	nd	0.38±0.01	1.03±0.07	0.94±0.07	0.88±0.08
		refrigerator	4.08±0.89	nd	1.05±0.10	nd	nd	0.18±0.01	093±0.08	1.78±0.12	0.92±0.04
	LC	fresh	12.8±1.6	nd	nd	nd	nd	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	nd	nd	nd	nd	nd
SnA	GC	fresh	nd	nd	1.78±0.13	nd	1.72±0.05	1.87±0.08	1.69±0.05	1.76±0.10	1.63±0.08
		refrigerator	nd	nd	1.61±0.06	nd	1.70±0.02	1.70±0.01	1.71±0.04	1.71±0.03	1.61±0.10
	LC	fresh	nd	nd	nd	nd	nd	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	nd	nd	nd	nd	nd
PrCA	GC	fresh	1.88±0.05	0.43±0.12	1.20±0.04	nd	1.50±0.05	nd	1.59±0.06	1.74±0.32	1.50±0.03
		refrigerator	0.18±0.11	0.25±0.07	0.88±0.05	nd	1.54±0.01	nd	1.63±0.02	1.36±0.23	1.55±0.04
	LC	fresh	nd	nd	nd	nd	nd	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 3.6 continous

CA	GC	fresh	21.2±1.6	nd	1.12±0.13	nd	nd	0.47±0.11	0.08±0.02	0.07±0.02	0.07±0.01
		refrigerator	10.6±0.6	nd	0.42±0.05	nd	nd	0.08±0.02	0.08±0.02	0.07±0.02	0.07±0.02
	LC	fresh	21.0±0.5	nd	nd	nd	nd	nd	nd	nd	nd
		refrigerator	10.5±0.6	nd	nd	nd	nd	nd	nd	nd	nd
Res	GC	fresh	nd	nd	nd	nd	nd	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	nd	nd	nd	nd	nd
	LC	fresh	nd	nd	nd	nd	nd	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	nd	nd	nd	nd	nd
GA	GC	fresh	nd	nd	363.1±3.3	23.6±2.0	nd	2.31±0.20	0.50±0.02	2.13±0.14	2.27±0.09
		refrigerator	nd	nd	220.6±1.9	11.3±2.0	nd	1.06±0.02	0.63±0.03	2.13±0.06	2.24±0.08
	LC	fresh	nd	nd	351.0±1.8	21.4±1.8	nd	nd	nd	nd	nd
		refrigerator	nd	nd	210.0±1.4	11.2±1.6	nd	nd	nd	nd	nd
Ka	GC	fresh	350.7±3.3	63.2±4.1	5.88±1.12	5.05±0.35	6.90±0.15	6.79±0.47	7.33±0.90	6.18±0.79	6.62±0.22
		refrigerator	58.4±4.1	42.2±1.4	4.10±0.32	2.45±0.16	6.51±0.03	3.30±0.09	7.28±0.07	68.0±0.06	6.80±0.05
	LC	fresh	340.3±6.1	59.6±2.3	6.9±0.5	nd	6.84±0.17	6.20±0.08	nd	6.06±0.11	6.82±0.39
		refrigerator	58.1±3.2	49.9±1.7	nd	nd	6.56±0.42	nd	nd	5.44±0.33	6.48±0.52
EC	GC	fresh	56.7±4.1	88.1±0.5	25.6±3.0	16.4±1.0	145.7±3.0	413.7±0.9	8.11±0.52	9.74±0.30	7.13±0.15
		refrigerator	17.6±1.8	68.0±1.6	6.88±1.26	4.11±0.38	131.5±4.1	31.2±6.7	7.90±0.06	8.12±0.05	7.24±0.07
	LC	fresh	56.1±1.3	86.4±2.0	27.4±1.9	14.2±1.5	139.9±2.0	411.3±1.8	8.97±0.12	9.01±0.18	6.86±0.28
		refrigerator	14.2±1.2	85.8±1.1	6.62±0.72	nd	128.6±2.1	34.2±1.7	7.33±0.30	nd	6.52±0.32
Q	GC	fresh	351.6±3.5	8.15±0.18	3.20±0.06	5.15±0.35	437.8±5.0	4.18±0.50	3.33±0.06	3.92±0.91	3.65±0.20
		refrigerator	63.4±2.7	7.65±0.47	2.64±0.11	2.81±0.18	420.0±2.6	3.34±0.16	3.36±0.03	3.97±0.28	3.28±0.14
	LC	fresh	362.5±3.5	9.25±0.53	nd	nd	402.9±3.7	nd	nd	nd	nd
		refrigerator	54.5±1.6	7.88±0.33	nd	nd	402.8±2.5	nd	nd	nd	nd
C	GC	fresh	24.5±3.4	48.0±0.5	13.6±3.0	12.24	170.5±2.4	465.6±0.8	12.8±0.3	16.5±1.4	10.4±1.9
		refrigerator	8.47±0.62	24.7±0.5	7.47±1.40	3.21±0.11	175.4±0.8	272.3±10.7	10.5±0.2	15.4±0.2	13.7±0.8
	LC	fresh	25.5±1.2	46.4±1.2	14.4±2.3	12.3±1.1	168.8±3.1	458.0±3.1	13.2±1.6	16.3±1.4	8.23±0.58
		refrigerator	7.31±0.65	54.5±4.7	6.96±0.64	202.4±2.0	142.3±2.8	271.7±2.5	9.98±0.13	nd	6.02±0.80
CGA	GC	fresh	nd	nd	4.70±0.54	nd	nd	3.43±0.37	4.74±0.50	4.14±0.39	nd
		refrigerator	nd	nd	4.25±0.09	nd	nd	1.82±0.06	nd	4.52±0.24	nd
	LC	fresh	nd	nd	6.07±0.05	nd	nd	nd	nd	nd	nd
		refrigerator	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 3.6 continous

R	GC	fresh	5.81±1.59	10.1±0.2	nd	nd	nd	nd	nd	2.31±0.18	nd
		refrigerator	nd	6.30±0.99	nd	nd	nd	nd	nd	2.25±0.31	nd
	LC	fresh	5.40±0.40	10.3±1.8	nd	nd	nd	nd	nd	nd	nd

Tablo 3.7 Recovery results obtained after determination of phenolic compounds in fruit juices by the proposed methods

		Orange		**Quince**		Grapefruit		Mandarin		Pear	
		$\bar{x}$±s (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**
CnA	GC	224.9±0.5	100.7/0.2	201.5±0.8	99.7/0.4	190.3±1.8	95.1/0.9	183.2±4.5	91.6/2.5	207.6±1.6	92.9/0.8
	LC	216.7±3.9	94.7/1.8	182.3±0.4	91.2/0.2	177.5±0.8	88.8/0.4	163.0±4.2	81.5/2.6	191.5±0.7	86.5/0.4
pCA	GC	236.7±0.5	100.1/0.2	199.8±7.3	98.8/3.7	195.4±4.6	96.6/2.4	235.5±0.8	98.4/0.3	192.8±3.6	95.7/1.9
	LC	207.2±6.1	89.2/3.0	170.2±2.1	85.1/1.2	174.0±2.8	87.0/1.6	216.6±4.7	91.7/2.2	180.6±3.7	90.3/2.1
VA	GC	289.3±0.9	97.3/0.3	210.7±1.0	97.2/0.5	307.8±2.5	98.5/0.8	229.9±0.8	97.2/0.4	542.1±10.2	98.1/1.9
	LC	267.9±3.4	90.5/1.3	196.2±8.0	91.9/4.1	285.3±0.5	91.870.4	211.5±2.1	91.2/1.0	468.2±3.4	93.1/0.7
FA	GC	222.8±6.5	97.9/2.9	188.7±1.1	94.3/0.6	183.0±1.4	91.5/0.8	217.7±3.8	93.8/1.8	187.6±1.9	93.4/1.0
	LC	206.2±6.3	89.2/3.1	164.9±6.4	82.4/3.9	169.0±1.4	84.5/0.8	205.2±4.0	89.2/1.9	168.6±1.0	84.3/0.6
SA	GC	190.7±1.0	90.0/0.5	189.7±1.9	94.8/1.0	189.4±8.7	94.2/4.6	187.0±4.2	93.5/2.3	185.8±1.7	92.9/0.9
	LC	194.2±0.3	91.2/0.2	173.6±2.9	86.8/1.6	182.0±4.2	91.0/2.3	176.5±4.9	88.2/2.8	170.0±1.9	84.9/1.1
SnA	GC	191.0±4.2	95.5/2.2	185.3±2.4	92.6/1.3	191.6±2.1	94.9/1.1	184.5±2.1	92.2/1.2	195.8±2.6	97.1/1.3
	LC	180.5±7.8	90.2/4.3	185.7±7.5	92.8/4.0	189.0±2.8	94.5/1.5	173.5±3.5	86.8/2.0	178.0±2.8	89.0/1.6
PrCA	GC	203.4±2.2	100.8/1.1	200.5±0.7	100.1/0.4	200.3±1.4	99.6/0.7	193.0±2.8	96.5/1.5	190.2±3.1	94.4/1.6
	LC	178.0±11.3	89.0/6.4	179.5±0.7	89.8/0.4	176.5±4.9	88.2/2.8	169.0±1.4	84.5/0.8	176.0±2.8	88.0/1.6
CA	GC	214.7±3.3	97.1/1.5	194.8±4.6	97.4/2.4	183.2±1.2	91.1/0.6	184.0±5.6	92.0/3.1	181.2±1.8	90.6/1.0
	LC	212.9±2.1	96.3/1.0	188.5±0.7	94.3/0.4	181.5±2.1	90.8/1.2	172.5±4.9	86.2/2.9	176.6±4.8	88.3/2.8
Res	GC	183.7±4.7	91.8/2.6	189.8±2.5	94.9/1.3	180.9±0.5	90.5/0.2	183.5±3.5	91.8/1.9	173.4±3.3	86.6/1.9
	LC	190.5±3.5	95.2/1.9	187.6±0.6	93.8/0.3	182.5±3.5	91.2/1.9	179.0±1.4	89.5/0.8	173.8±3.9	86.8/2.2
GA	GC	180.7±4.2	90.3/2.3	180.5±0.8	90.2/0.4	487.2±4.6	86.5/0.9	210.2±1.2	93.9/0.6	193.2±2.6	96.6/1.3
	LC	172.0±5.7	86.0/3.3	168.7±8.0	84.3/4.7	471.5±3.5	85.6/0.8	199.2±1.1	89.9/0.6	177.2±1.3	88.6/0.8
Ka	GC	554.0±1.4	100.6/0.3	266.7±2.4	100.2/0.9	203.0±1.4	98.6/0.7	203.0±1.4	99.0/0.7	197.1±9.6	95.3/4.8
	LC	519.6±7.1	98.8/1.4	251.9±17.5	97.1/6.9	20.7±2.0	98.4/1.0	192.0±7.1	96.0/3.7	192.0±1.9	92.8/1.0
EC	GC	250.5±3.5	97.6/1.4	283.0±2.8	98.2/1.0	219.1±1.3	97.1/0.6	210.0±1.5	97.1/0.7	339.4±1.0	98.2/0.3
	LC	250.7±0.5	97.8/0.2	280.7±0.5	98.0/0.2	222.4±1.9	97.8/0.9	210.0±1.4	98.1/0.6	326.6±3.6	96.1/1.1
Q	GC	540.2±1.5	99.8/0.3	209.0±1.4	101.7/0.7	201.7±0.5	99.2/0.2	199.3±1.0	97.1/0.5	627.6±4.9	98.4/0.8
	LC	561.9±9.2	98.1/1.6	204.5±3.1	97.7/1.5	190.5±5.0	95.2/2.6	181.0±2.8	90.5/1.6	519.0±2.5	86.1/4.9
C	GC	219.4±1.1	97.7/0.5	240.7±0.5	97.0/0.2	206.2±1.1	96.6/0.5	207.0±1.6	97.5/0.8	363.0±4.3	97.9/1.2
	LC	218.5±1.5	96.8/0.7	236.8±2.6	96.1/1.1	208.4±2.0	97.2/1.0	202.4±2.0	95.4/1.0	354.0±2.8	95.9/0.8
CGA	GC	181.0±2.8	90.5/1.6	189.0±1.4	94.5/0.8	198.0±2.8	96.7/1.4	188.5±2.1	94.2/1.1	189.5±0.8	94.8/0.4
	LC	180.5±4.9	90.2/2.7	173.8±6.8	86.9/3.9	182.0±4.2	88.3/2.3	172.5±3.5	86.2/2.0	182.2±2.2	91.1/1.2

Table 3.7 continous

R	GC	176.4±1.9	85.7/1.1	182.5±3.5	86.9/1.9	169.2±1.2	84.6/0.7	174.0±2.8	87.0/1.6	169.2±1.7	84.6/1.0
	LC	182.5±3.5	88.8/1.9	183.8±2.6	87.4/1.4	172.5±3.5	86.2/2.1	172.0±1.4	86.0/0.8	167.5±1.5	83.8/0.9

nd: not detected; RR: Relative recovery; spiked conc. = 200 $\mu g\ L^{-1}$, n = 3

Tablo 3.8 Recovery results obtained after determination of phenolic compounds in fruit juices by the proposed methods

		Pomegranate		**Fuji apple**		**Starking apple**		**Golden apple**	
		$\bar{x}$±s (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**	**$\bar{x}$±s** (ug L^{-1})	**RR/RSD (%)/(%)**
CnA	GC	201.5±1.1	99.7/0.6	184.2±1.3	92.1/0.7	188.2±2.6	91.7/1.4	184.4±2.8	90.9/1.5
	LC	170.5±2.1	85.3/1.2	170.5±0.8	85.3/0.4	167.4±2.4	83.7/1.4	170.6±0.8	85.3/0.5
pCA	GC	199.8±2.1	98.8/1.1	194.7±1.4	96.6/0.7	196.6±1.9	97.5/1.0	189.5±2.1	94.1/1.1
	LC	181.8±2.6	90.9/1.4	177.5±2.1	88.8/1.2	180.4±4.1	90.2/2.3	183.6±2.3	91.8/1.2
VA	GC	210.7±2.2	97.2/1.2	202.6±0.8	97.5/0.4	202.7±0.5	96.9/0.3	200.6±0.7	96.9/0.3
	LC	185.4±7.7	92.7/4.1	188.9±2.7	91.0/1.4	191.3±1.8	91.3/0.9	189.0±1.4	91.6/0.8
FA	GC	188.7±2.3	94.3/1.2	190.0±1.9	94.6/1.0	190.2±2.3	94.7/1.2	184.9±1.0	92.1/0.5
	LC	164.9±1.4	82.4/0.8	174.8±5.9	87.4/3.4	169.0±4.2	84.5/2.5	169.1±1.1	84.6/0.6
SA	GC	189.7±6.1	94.8/3.2	190.6±3.7	94.8/1.9	185.0±4.2	92.1/2.3	183.6±3.8	91.4/2.0
	LC	168.0±0.4	84.0/0.3	178.0±2.8	89.0/1.6	172.4±3.2	86.2/1.8	169.5±0.7	84.8/0.4
SnA	GC	185.3±2.6	92.6/1.4	188.7±0.9	93.5/0.5	190.8±0.7	94.5/0.4	194.8±0.3	96.6/0.3
	LC	182.3±1.8	91.1/1.1	177.2±4.5	88.6/2.5	170.2±2.6	85.1/1.5	175.8±2.6	87.9/1.5
PrCA	GC	200.5±2.6	100.1/1.4	188.5±0.9	93.5/1.1	185.5±3.5	92.0/1.9	188.0±1.4	93.3/0.7
	LC	173.9±0.4	86.9/0.2	173.8±4.5	86.9/2.6	178.6±3.7	89.3/2.1	179.6±2.4	89.8/1.3
CA	GC	194.8±2.1	97.4/1.2	189.0±1.4	94.5/0.8	184.4±1.9	92.1/1.0	186.7±1.7	93.4/0.9
	LC	179.0±1.3	89.5/0.8	179.0±1.4	89.5/0.8	182.2±1.7	91.1/0.9	177.9±1.8	88.9/1.0
Res	GC	189.8±1.1	94.9/0.7	178.0±0.1	89.0/1.6	173.6±4.6	86.8/2.6	173.7±3.2	86.8/1.9
	LC	173.7±2.4	86.8/1.3	183.5±2.1	91.8/1.2	177.7±5.1	88.8/2.8	175.2±1.7	87.6/1.0
GA	GC	180.5±3.4	90.2/1.7	195.8±4.5	97.6/2.3	195.6±7.6	96.8/3.9	200.0±1.4	98.8/0.7
	LC	173.1±2.7	86.6/1.6	181.0±2.8	90.5/1.6	173.5±3.5	86.8/2.1	176.0±1.4	88.0/0.8
Ka	GC	206.7±0.1	100.2/0.1	199.0±4.2	96.0/2.1	196.0±4.2	95.1/2.2	193.7±1.8	93.7/0.9
	LC	190.5±0.7	92.4/0.4	184.0±1.4	92.0/0.8	188.6±2.4	91.5/1.3	189.2±1.7	91.5/0.9
EC	GC	601.0±3.6	98.2/0.7	204.6±0.8	98.2/0.4	203.0±1.4	96.8/0.8	201.2±0.3	97.1/0.2
	LC	584.9±23.1	95.7/3.9	201.6±2.1	96.4/1.0	201.0±3.3	96.0/1.6	202.2±0.4	97.8/0.2
Q	GC	209.0±0.6	101.7/0.3	193.7±1.3	95.3/0.7	199.8±1.6	98.0/0.8	200.0±1.9	98.2/1.0
	LC	174.1±2.7	87.1/1.5	182.0±7.1	91.0/3.9	164.9±5.7	82.5/3.5	168.6±2.3	84.3/1.4
C	GC	658.7±1.3	97.0/0.4	207.5±1.5	97.5/0.7	207.9±2.2	96.0/1.1	201.4±1.3	97.1/0.6
	LC	639.1±8.7	97.1/1.4	204.3±1.1	95.8/0.5	210.5±0.8	97.4/0.4	200.1±1.3	96.1/0.6
CGA	GC	189.0±3.1	94.5/1.6	199.0±2.8	97.2/1.4	200.5±2.1	98.2/1.1	192.8±2.1	96.471.1
	LC	186.2±2.5	93.1/1.3	1178.4±0.1	89.2/2.1	173,6±4.7	86.8/2.7	180.7±0.9	90.4/0.5
R	GC	182.5±4.8	86.9/2.8	169.4±1.9	84.7/1.1	174.3±1.9	86.2/1.1	172.3±3.3	86.2/1.9
	LC	170.0±1.7	85.0/1.0	171.4±2.8	85.7/1.6	174.0±1.4	87.0/0.8	170.0±1.4	85.0/0.8

nd: not detected; RR: Relative recovery; spiked conc. = 200 $\mu g\ L^{-1}$, n = 3

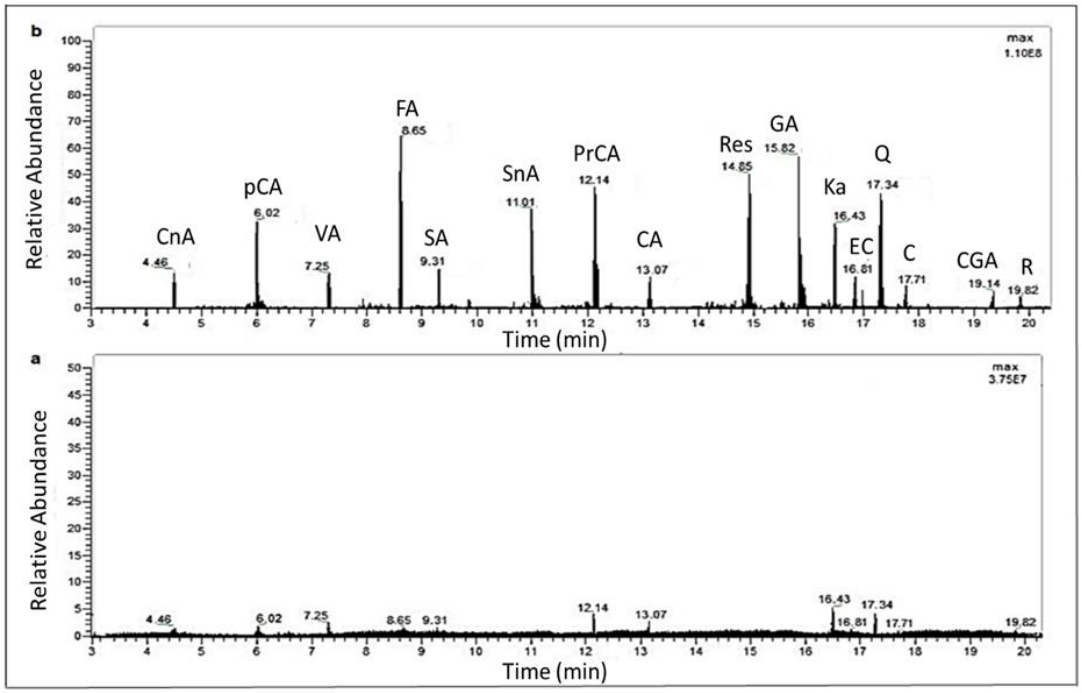


Figure 3.28 Total ion chromatograms in SIM mode of orange juice sample with 16 phenolic compounds added at 200 µg L^{-1} concentration using KSF-APBI-BF_4 fiber by SPME/GC-MS method

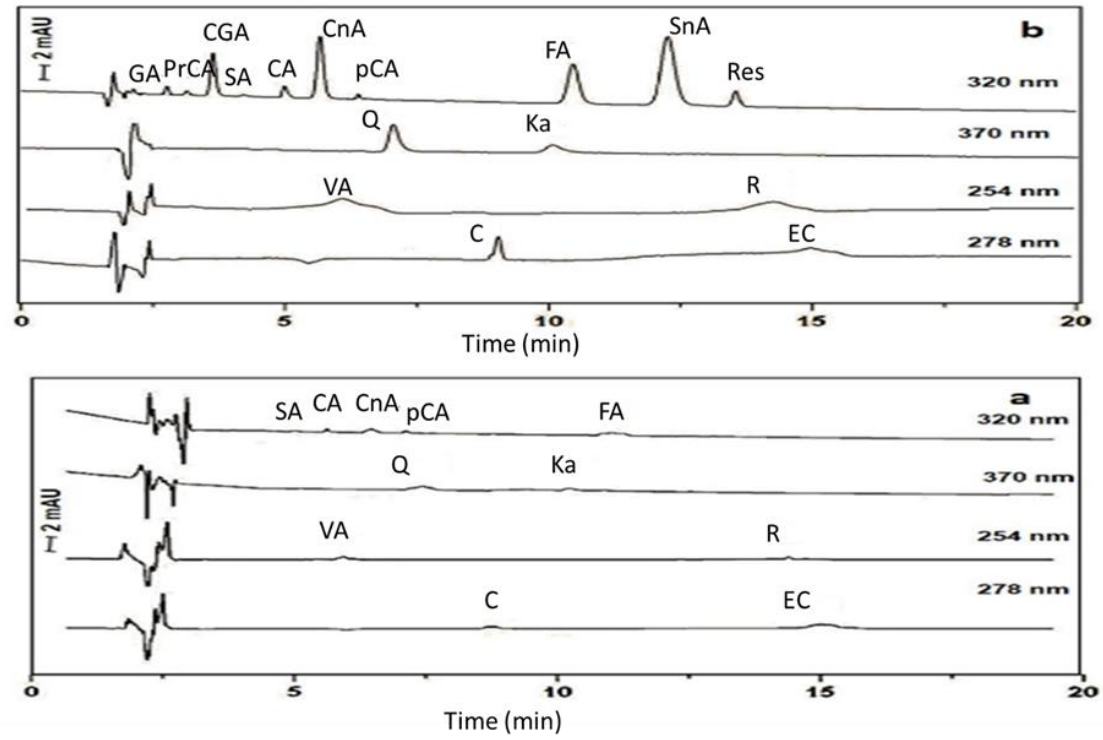


Figure 3.29 LC chromatograms of a) orange juice sample using KSF-APBI-BF_4 fiber by SPME-LC method, b) orange juice with 16 phenolic compounds added at 200 µg L^{-1} concentration

3.5 Comparison of the Lab-made Fibers with the Previous SPME Studies

The proposed SPME-GC-MS method using GO-APMI-TFSI coated fiber has better or comparable analytical performance than the previous studies based on SPME methods for the analysis of phenolic compounds, especially in wine samples, in terms of linear ranges, and LODs (Table 3.8) (Aresta, Cotugno, Massari, & Zambonin, et al. 2018; Mirnaghi, Mousavi, Rocha, & Pawliszyn, 2013; Vinas, Campillo, Martinez-Catillo, & Hernandez-Cordoba, 2009; Vinas, Campillo, Martinez-Catillo, & Hernandez-Cordoba, 2008; Cai, Koziel, Dharmadhikari, & van Leeuwen, 2009; Chen, & Huang, 2017, Citova, Sladkovsky, & Solich, 2006). In terms of optimization conditions, it can be said that the proposed SPME method has similar or lower stirring rate, lower extraction temperature and shorter extraction time than the methods given in Table 3.9. In addition, more phenolic compounds were analyzed by SPME-GC-MS using GO-APMI-TFSI fiber after derivatization on the fiber.

Previously published studies about analysis of phenolic compounds using SPME method with different coating materials coupled to chromatographic detection were compared with the proposed methods (Vinas, Campillo, Martinez-Catillo, & Hernandez-Cordoba, 2009; Pei, & Huang, 2016; Citova, Sladkovsky, & Solich, 2006; Vinas, Campillo, Martinez-Catillo, & Hernandez-Cordoba, 2008; Cai, Koziel, Dharmadhikari, & van Leeuwen, 2009; Mirnaghi, Mousavi, Rocha, & Pawliszyn, 2013; Che, & Huang, 2017; Aresta, Cotugno, Massari, & Zambonin, 2018). As with the SPME based methods, the samples were diluted with water in the methods given in the relevant table, except for the study of Cai et al. (Cai, Koziel, Dharmadhikari, & van Leeuwen, 2009). In this study, two PA fibers were used through the experiments. Each one was used for 30 injections because the wine samples were used directly and accumulation on fiber occurred. As seen in the given methods except for Mirnaghi and coauthors (2013) and Chen and coauthors (2017). In the studies given in Table 3.8, the confirmation of the methods was achieved by spiking analyte standards to real samples. All given studies presented similar limit of detection values and working range with the developed methods based on KSF-ionic liquid coated fibers. Better analytical performance was obtained with KSF-APBI-BF4 fiber from the prepared

KSF-containing fibers, and it showed a similar or better performance than other methods. In addition, it is as good at recoveries as any other work.

Table 3.9 Comparison of the coating material, the sample type, the linear ranges and LODs of the developed methods with the previous methods

Detection method	Material	PCs	Sample	Linear range (ug L^{-1})	LOD (ug L^{-1})	Ref.
GC/FID[1,2]	PA	pCA, SA PrCA, FA, CA, GA	Test solution	2.2-354.4	0.01-1.77	(Citova, Sladkovsky, & Solich, 2006)
LC/DAD	MMF	pCA, FA, HBA SA	Fruit juice	1-200 2-200	0.17-0.30 0.57	(Pei, & Huang, 2016)
LC/FLD	CW/TPR	Res	Wine, must & fruit juice	5-150	2	(Vinas, Campillo, Martinez-Catillo, & Hernandez-Cordoba, 2008)
GC-MS	PA	Res	wine	10-5000	7.08	(Cai, Koziel, Dharmadhikari, & van Leeuwen, 2009)
GC-MS	PA	Res	Wine & grape	1-150	0.09	(Vinas, Campillo, Martinez-Catillo, & Hernandez-Cordoba, 2009)
LC/MS-MS	PS-DVB-PAN	CA Res	Wine, berry & grape	1.5-500 5-500	0.5 1.5	(Mirnaghi, Mousavi, Rocha, & Pawliszyn, 2013)
LC/DAD	PIL-MIP	CA FA	Fruit juice & beer	0.1-200 0.05-200	0.019-0.024 0.011-0.042	(Chen, & Huang, 2017)
LC/DAD	PA	Res	Wine, spirit & grape juice	0.1-500	0.4	(Aresta, Cotugno, Massari, & Zambonin, 2018)
GC-MS	GO-APMI-TFSI	CnA, SA, SnA, PrCA, Res, Ka, Q, CGA, pCA, FA, CA, GA	Wine	0.1-1000	0.02-0.05 0.02-0.1	This study
GC-MS	KSF-APBI-BF_4 KSF-APBI-Br	CnA, SA, SnA, PrCA, Res, Ka, Q, CGA pCA, FA, CA, GA	Fruit juices	0.02-1000 0.1-1000	0.01 0.05	This study
LC/DAD	KSF-APBI-BF_4 KSF-APBI-Br	VA, C, EC, R		5-1000 5-1000	2.5 2.5	

PS-DVB-PAN: polystyrene-divinylbenzene-polyacrylonitrile, PIL/MIP: poly (ionic liquid)-molecularly imprinted polymer, MMF: multiple monolithic fiber, HBA: 4-hydroxybenzoic acid.

CHAPTER FOUR
CONCLUSIONS

In the study, ionic liquid-based materials containing graphene oxide and montmorillonite clay were prepared for the separation and preconcentration of phenolic compounds from different kinds of samples before their chromatographic determination. They were used in solid phase microextraction fiber coating, and SPME methods were developed. For this purpose, APMI-Br, APMI-BF_4, APMI-TFSI, APBI-Br, APBI-BF_4 and APBI-TFSI ionic liquids were synthesized. The synthesized ionic liquids structures were characterized by FTIR and TG. GO-APMI-BF_4 and GO-APMI-TFSI and KSF-APMI-Br, KSF-APMI-BF_4, KSF-APMI-TFSI, KSF-APBI-Br, KSF-APBI-BF_4 and KSF-APBI-TFSI coated SPME fibers prepared. The selectivity of GO-APMI-BF_4 and GO-APMI-TFSI coated SPME fibers against 12 phenolic compounds was investigated. It was determined that the extraction efficiency of GO-APMI-TFSI coated fiber was better. The selectivity of 6 KSF-IL coated fibers against 16 phenolic compounds (VA, C, EC and R; they are not included in the study with GO since they were supplied later) were examined. It was determined that the extraction efficiencies of KSF-APBI-Br and KSF-APBI-BF_4 coated fibers were better. For this reason, only fibers with high efficiency were characterized by FTIR, XRD, TG and SEM methods.

Under the optimized conditions (extraction temperature 30 °C, extraction time 30 minutes, 5 minutes desorption), the SPME method coupled with GC-MS after derivatization on the fiber (30 minutes) with GO-APMI-TSFI coated fiber have been successfully applied to wine samples (red and white wines and black mulberry, melon, red plum and blueberry fruit wines) for the extraction and determination of phenolic compounds. To check accuracy of the developed method, standard phenolic mixture solution was added to the samples, and high recoveries were obtained. High extraction efficiency of phenolic compounds was obtained with GO-APMI-TFSI coated fiber at pH 8. In addition to the electrostatic interactions between the hydrophobic TFSI ion fiber and phenolic compounds in anionic form at the pH studied, π-π and dipole-dipole interactions can be mentioned within GO and IL and phenolic compounds. Therefore, GO-APMI-TFSI coated fiber can be considered as suitable SPME fiber for extraction

of phenolic compounds from various food samples. The developed fiber showed long-term stability, high durability, good thermal behavior, and high fiber-to-fiber reproducibility without a reduction in extraction performance after more than 150 extraction cycles. The prepared fiber presented a large working range, low detection limit and good reproducibility in the analysis of phenolic compounds in different kinds of wine samples.

Direct immersion SPME coupled with GC-MS and SPME coupled with LC methods were proposed for polar phenolic compounds using KSF-APBI-Br and KSF-APBI-BF_4 coated fibers. Phenolic compounds were extracted successfully KSF clay with APBI-X (X: Br, BF_4, TFSI) based and hydrophilic anion (Br, BF_4) ionic liquids than the APMI-X ionic liquids at pH 4. The possible interactions can be intermolecular forces such as π-π interactions, H-bonding and electrostatic interactions. To show the applicability of the proposed method using KSF-APBI-BF_4 coated fiber, samples of fresh fruit juices (orange, grapefruit, tangerine, quince, pear, pomegranate, apple) was used and standard phenolic mixture solution at different concentrations were spiked and high recovery was obtained. The prepared SPME fibers with clay-ionic liquid based materials presented high thermal behavior, good durability, good thickness, long service life (>160). The sensitivity of LC was found lower than the GC-MS method.

When the GO and KSF clay-coated fibers were compared, the lifetimes of both groups of fibers were close to each other. Extraction affinities for phenolic compounds were high in both species. In addition, the extraction efficiencies of the lab-made fibers with the ionic liquid based graphene oxide and KSF clay were much higher than the commercial fibers studied (CW/PEG, PA, DVB/CAR/PDMS, and CAR /PDMS). In both types of fibers, environmentally friendly ionic liquids were used in the fiber coating. Inexpensive, abundant KSF-montmorillonite clay has been a more economical coating material for fiber preparation than graphene-based materials and carbon nanotubes.

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