SPENT TEA LEAVES AS AN ADSORBENT FOR MICRO-SOLID PHASE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DIFFERENT MATRICES

NAZZATUL ATIRAH BT MOHD NAZIR

FACULTY OF SCIENCE

UNIVERSITI MALAYA

KUALA LUMPUR

2021

SPENT TEA LEAVES AS AN ADSORBENT FOR MICRO-SOLID PHASE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DIFFERENT MATRICES

NAZZATUL ATIRAH BT MOHD NAZIR

**DISSERTATION SUBMITTED IN FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE**

**DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR**

2021

UNIVERSITI MALAYA

ORIGINAL LITERATURE WORK DECLARATION

Name of Candidate: **NAZZATUL ATIRAH BTE MOHD NAZIR**

Matric No: **SMA180023 / 17007179/2**

Name of Degree: **MASTER OF SCIENCE**

Title of Project Paper/Research Report/Dissertation/Thesis (“this Work”):

SPENT TEA LEAVES AS AN ADSORBENT FOR MICRO-SOLID PHASE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DIFFERENT MATRICES

Field of Study: **ANALYTICAL CHEMISTRY**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every right in the copyright to this Work to the University of Malaya (“UM”), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work, I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate’s Signature Date: 10/2/2021

Subscribed and solemnly declared before,

Witness’s Signature Date: 10/2/2021

Name:

Designation:

SPENT TEA LEAVES AS AN ADSORBENT FOR THE MICRO-SOLID-PHASE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN DIFFERENT MATRICES

ABSTRACT

Food and water may be contaminated by hazardous pollutants, one of is a polycyclic aromatic hydrocarbon (PAHs), where 16 of pollutant in this group listed as a priority pollutant by Environmental Protection Agency (EPA). Thus, analysis of food contaminations is crucial for consumer safety. Therefore, a simple, sensitive, and miniaturize sample preparations are needed. In this study, spent tea leaves (STL), a waste from brewed tea, *Camellia sinensis* was utilized as novel micro-solid phase extraction (μ-SPE) sorbent for the determination of polycyclic aromatic hydrocarbon (PAHs) for the first time. It was of interest that spent tea leaves (STL) might serve as a suitable sorbent due to the presence of a variety of functional groups like lignin, cellulose, hemicellulose, and polyphenols that naturally can interact and adsorb the hydrophobic PAHs from real samples. STL was characterized by Fourier transform infrared spectroscopy (FT-IR), field emission scanning electron microscope (FESEM), and energy dispersive x-ray analysis (EDX). Besides that, the interactions between PAHs and STL were proven by FT-IR, FESEM, and EDX analysis. Moreover, a key parameter in the extraction efficiency of STL based μ- SPE, such as eluent type and volume, the dosage of sorbent, extraction and desorption time, and volume of the sample was examined. Finally, an effective, environmentally friendly, and economic STL based tea bag filter as porous membrane protected STL-μ-SPE method for the determination of five types of PAHs (Flu, Flt, Pyr, Chr, and BaP) was developed and successfully applied in the analysis of water, rice, orange, and apple juice samples.

Under the optimized conditions, the matrix matched calibration curves were linear in the range of 50 ng mL^{-1} to 1000 ng mL^{-1} and the coefficient of determinations (R^2) found to be between 0.9947 and 0.9983. The LOD and LOQ of liquid and solid sample is in range of 8.47-55.95 ng mL^{-1}, 2.98-30.22 µg kg^{-1} and 28.23-186.54 ng mL^{-1}, 9.04-91.59 µg kg^{-1}. While the intra-day and inter-day precision (n=6) were found to be between 5.23 % to 7.76 % and 6.90 % to 10.86 %, respectively. The recovery values in the real samples are between 88.0 % and 111.4 % and its RSDs (n=3) from 1.0 % to 9.8 %. The present method found to be fast, sensitive, cost-effective, reproducible, and this work also introduces a new application method of the agricultural crop in the determination and quantification of PAHs in real samples analysis.

Keywords: Polycyclic aromatic hydrocarbons (PAHs), porous membrane protected micro- solid-phase extraction (µ-SPE), spent tea leaves (STL), low-cost adsorbent.

DAUN TEH YANG TELAH DIGUNAKAN SEBAGAI PENJERAP UNTUK PENGEKSTRAKAN MIKRO-FASA-PEPEJAL POLISIKLIK AROMATIK HIDROKARBON (PAH) DALAM MATRIK YANG BERBEZA

ABSTRAK

Makanan dan air mungkin dicemari oleh bahan pencemar organic yang berbahaya, salah satu daripadanya ialah polisiklik aromatik hidrokarbon (PAHs), dimana 16 daripadanya disenaraikan sebagai bahan pencemar utama oleh Agensi Pelindungan Alam Sekitar (EPA). Oleh itu, analisa pencemaran makanan adalah penting untuk keselamatan consumer. Oleh kerana itu, penyediaan sampel yang ringkas, peka, dan miniatur diperlukan. Dalam kajian ini, daun teh yang telah digunakan (STL) iaitu sisa dari teh yang telah dibancuh, *Camellia sinensis* telah digunakan sebagai penjerap baru μ-SPE untuk penentuan PAH buat pertama kalinya. STL boleh menjadi penjerap yang sesuai kerana ia mengandungi pelbagai kumpulan fungsi seperti lignin, sellulosa, hemisellulosa, dan polifenol yang secara semula jadinya boleh berinteraksi dan menjerap PAH yang hidrofobik dari sampel sebenar. STL dicirikan oleh spektroskopi inframerah fourier transformasi (FT-IR), mikroskopi pengimbas pelepasan elektron (FESEM), dan spektroskopi sinar-X dispersif tenaga (EDX). Selain itu, hubungan antara PAH dan STL juga dibuktikan berdasarkan analisa FT-IR, FESEM, dan EDX. Selain itu, parameter penting dalam kecekapan pengekstrakan STL-μ-SPE seperti jenis dan isipadu eluen, dos penjerap, masa pengestrakan dan penyerapan, dan isipadu sampel diperiksa. Akhir sekali, STL yang dilindungi oleh penapis beg teh sebagai membran berliang μ-SPE yang berkesan, mesra alam sekitar, dan menjimatkan kos untuk penentuan lima jenis PAH (Flu, Flt, Pyr, Chr, dan BaP) telah dimajukan dan berjaya diaplikasikan untuk analisa sampel air, beras, jus oren, dan jus epal. Dalam keadaan optimum, lengkung penentukuran matrik padanan telah menunjukkan kelinearan dalam julat 50 ng mL^{-1} hingga 1000 ng mL^{-1} dan pekali penentuan (R^2) di antara 0.9947 dan 0.9983. LOD dan LOQ untuk sampel cecair dan pepejal adalah dalam julat 8.47-55.95 ng mL^{-1}, 2.98-30.22 µg kg^{-1}, dan 28.23-186.54 ng mL^{-1}, 9.04-91.59 µg kg^{-1}. Manakala, ketepatan intra-hari dan inter-hari (n=6) adalah di antara 5.23 %-7.76 % and 6.90 %-10.86 %. Nilai kebolehdapatan semula sampel sebenar adalah antara 88.0 % dan 111.4 % dan RSDs (n=3) dari 1.0 % hingga 9.8 %. Kaedah ini didapati cepat, sensitif, menjimatkan kos, boleh dihasilkan semula, dan kajian ini juga memperkenalkan kaedah baru mengaplikasikan tanaman pertanian dalam penentuan dan pengukuran PAH dalam menganalisis sampel sebenar.

Kata kunci: Hidrokarbon aromatik polisiklik (PAH), pengekstrakan fasa mikro-pepejal terlindung berliang (µ-SPE), daun teh yang telah digunakan (STL), penyerap kos rendah.

AKNOWLEDGEMENTS

First and foremost, Alhamdulillah thank you Allah for helping and allowing me to complete this dissertation. Without opportunity given by the University of Malaya this work would have been impossible. Besides, big thank for the sincere inspiration and constant support given throughout this project by my supervisors, Associate Prof. Dr. Sharifah Binti Mohamad and Dr. Muggundha a/l Raoov. They provided me invaluable supervision, guidance, and sincere advice. I appreciate all their contributions of time, support, and ideas to complete my study.

Besides, profound gratitude to my family, fore mostly my mother, Puspawate Binti Mohd Yusuff, and my father, Mohd Nazir Bin Razak, for their support and encouragement throughout this despicable journey. Other than that, sincere thanks for all my lab mates and staff of the Department of Chemistry for their help and supports.

Lastly, thanks to the Faculty of Science, University of Malaya for providing funds in the research grant (GPF049B-2018) and a conducive environment for me to conduct the study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOL USED

<LOQ : Lower than limit of quantification

μ- SPE : Micro-solid-phase extraction

ATR : Attenuated total reflection

BaA : Benzo[a]anthracene

BaP : Benzo(A)pyrene

BbF : Benzo[b]fluoranthene

Chr : Chrysene

DLLME : Dispersive liquid-liquid micro-extraction

d-SPE : Dispersive solid-phase extraction

EBT : Eriochrome Black T

EDX : Energy Dispersive X-Ray Analysis

EPA : Environmental Protection Agency

FESEM : Field Emission Scanning Electron Microscope

Flt : Fluoranthene

Flu : Fluorene

FT-IR : Fourier Transform Infrared Spectroscopy

GC-FID : Gas chromatography- flame ionization detector.

GC-MS : Gas chromatography-mass spectrometry

GC-MS/MS : Tandem Mass Spectrometry

HMW : High molecular weight

HPLC : High performance liquid chromatography

HPLC-MS/MS : High performance liquid chromatography–tandem mass

HPLC-UV : High performance liquid chromatography- ultraviolet.

LC-MS/MS	: Liquid chromatography-tandem mass spectrometry,
LLE	: Liquid-liquid extraction
LMW	: Low molecular weight
LOD	: Limit of detection
LOQ	: Limit of quantification
LPME	: Liquid phase micro-extraction
MB	: Methylene blue
MIP	: Molecular imprinted polymer
MO	: Methyl Orange
MSPE	: Magnetic solid-phase extraction
nd	: Not detected
PAHs	: Polycyclic aromatic hydrocarbon
PE	: Polyethylene
PF	: Pre-concentration factor
Phe	: Phenanthrene
PP	: Polypropylene
Pyr	: Pyrene
R^2	: Coefficient of determinations
RB5	: Reactive Black 5
RSD	: Relative standard deviation
SD	: Standard deviation
SPE	: Solid-phase extraction
SPME	: Solid-phase micro-extraction
STL	: Spent tea leaves
STL-µ-SPE	: Spent tea leaves-micro-solid-phase extraction

TEQ : Toxic Equivalent Factor

US EPA : US Environmental Protection Agency

CHAPTER 1 : INTRODUCTION

1.1 Background of study

In recent years, organic pollutant contaminations become big issues for human beings. One of them is polycyclic aromatic hydrocarbons (PAHs), a huge group of persistent organic pollutants and most of it proved to have carcinogenicity, teratogenicity, and mutagenicity character which posing a tremendous threat to human health (Sun et al., 2019). 16 of them listed as priority pollutants by the US Environmental Protection Agency (US EPA) (US EPA, 2014). They released into the environment from natural and anthropogenic sources (Karyab et al., 2013), derived from incomplete combustion or pyrolysis of organic materials (Simoneit, 2002). As the population on the earth increases, the global emission of PAHs will proportionally increase (Hafner et al., 2005) which eventually makes PAHs contamination more threatening to human beings.

Humans exposed to PAHs via air and drinking water, but mostly through the intake of food (Vyskocil et al., 2000). Dietary ingestion was proved to be a major pathway of PAHs exposed to a person who did not smoke and did not work in PAHs exposure environment (Alomirah et al., 2011; Menzie et al., 1992). The food contaminated by PAHs mainly arose from production practices and environmental contamination. Short exposure to a high concentration of PAHs may cause nausea, confusion, and maybe worse for asthmatics people as it may disable their lung function. Hazardous effect on humans makes it is crucial to detect and determine PAHs concentration in the environment. Due to this, EPA sets a legal maximum level limit of BaP in drinking water to be 0.2 μg/L by complementing Safe Drinking Water Act (Zelinkova & Wenzl, 2015).

Consequently, their determination in environment and food samples has become an important topic in analytical chemistry. However, in most cases, the amounts of PAHs are usually found to be below the detection limit of many analytical techniques, and matric interference comes along with their determination (Hu et al., 2014). Hence, preliminary separation and pre-concentration of these compounds are often required to achieve accurate, sensitive, and reliable results by an analytical method. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) is well- known analytical sample preparation technique which developed to determine PAHs in real samples but due to the consumption of a large amount of chemicals (synthetic sorbents and organic sorbent), extended and multistep extraction procedures, difficulty in phase separations, and requirements of large volumes of samples are some major drawbacks of these techniques which makes LLE and SPE less popular. The recent regulations concerning the excessive use of organic solvents limit the scope of LLE and SPE as a sample preparation technique. In order to minimize their impact on workers and the environment, it is, therefore, highly desirable to reduce the number of organic solvents and other chemicals used during the sample preparation. Then after two decades of rapid evolution in this area of research, different techniques have been introduced emphasizing miniaturization, simplification, and automation of extraction mechanism. Hence, the different miniaturized technique such as solid-phase micro-extraction (SPME) (Aguinaga et al., 2007), dispersive solid-phase extraction (d-SPE) (Nasrollahpour et al., 2017), liquid phase micro-extraction (LPME) (Zanjani et al., 2007), and dispersive liquid-liquid micro-extraction (DLLME) (Zhao et al., 2009) have been developed and introduced.

Other than that, porous membrane protected micro-solid-phase extraction (μ-SPE) also was developed as an alternative to SPE (Basheer et al., 2006). This method differs from conventional SPE as it is done in a much smaller scale, simpler procedures where the device can be separated from matrix solution easily, it also has a high resistance to ‘dirty sample’, as the adsorbent is protected in the membrane (Basheer et al., 2006) and the clean-up and pre-concentration steps happen in single steps (Naing et al., 2016a). Numerous researches done by utilizing this technique, they varied type of adsorbent, sample matrix, and even type of membrane used depends on the target analyte they were targeting (Basheer et al., 2006; Basheer et al., 2009; Kanimozhi et al., 2011). First and foremost, it was essential to select and produce an appropriate sorbent to effectively extract the target analyte by the μ-SPE technique, recently, special consideration has been paid for the usage of available-in-nature, abundant, and eco-friendly adsorbents to supplant the conventional and costly adsorbent (Jawad et al., 2016). Agricultural waste could be potentially used as adsorbents as it represents unused resources, low-cost, generally accessible, and environmentally friendly. Agricultural by-product represents a promising and economic alternative as adsorbents because of the unique chemical composition, high reactivity, and excellent selectivity towards metal and organic pollutants, which results from the high content levels of cellulose, hemicellulose, and lignin with abundant reactive groups (Dai et al., 2018).

One of the well-known wastes used as adsorbent is spent tea leaves (STL), *Camellia sinensis* is dried and processed to produce tea (Mokgalaka et al., 2004). After the tea is brewed, the waste is called spent tea leaves (STL). Previously, STL usually uses for the removal of dyes and metal ions. For instance, the removal (MB) dye (Hameed, 2009), crystal violet dye (Bajpai & Jain, 2010), azo dyes (Zuorro et al., 2013), lead (II) (Lavecchia et al., 2010), and chromium (VI) (Malkoc & Nuhoglu, 2006) from water samples. Besides, the interaction of STL with organic pollutants was investigated by Lin et al. (2007). The sorption of phenanthrene proved happened and concluded that STL can strongly sorb the hydrophobic organic compound and can be potential sorbent for PAHs if it is properly handled. It is possible due to STL chemical content: 6.5% of lignin, cellulose, and hemicellulose which make it suitable for the adsorption of organic pollutants, PAHs (Huang et al., 2006). Moreover, tea also contains 80.5 to 134.9 mg of polyphenols (Yashin et al., 2015). Polyphenols contain lots of aromatic rings that can have hydrophobic interaction with aromatic rings of PAHs, thus facilitate the adsorption of PAHs onto tea surfaces.

In this study, STL-μ-SPE technique developed to determine five types of PAHs (fluorene (Flu), fluoranthene (Flt), pyrene (Pyr), chrysene (Chr), and benzo(a)pyrene (BaP)) in water, rice, orange juice, and apple juice. This proposed technique is cost-efficient, bio-degradable; uses a lesser solvent, less time, and much simple than other methods. Besides that, to reduce cost, tea bag filters made up of polyethylene (PE) and polypropylene (PP) were used instead of buying an expensive industrial made PE and PP porous membrane. Other than that, it also able to determine the trace level of PAHs, do the pre-concentration, and sample clean up in a single step. Extraction parameters like type of eluent, the dosage of adsorbent, extraction time, desorption time, the volume of sample, and volume of eluent are optimized. This technique also tested for its performances and its applicability to real samples. Only then, under optimum condition, STL- μ-SPE was applied on water, rice, orange, and apple juice samples.

1.2 Objectives of research

The objectives of this study are as follow:

1) To prepare and characterize spent tea leaves (STL) as an adsorbent.
2) To develop and validate the STL- μ- SPE technique for the determination of PAHs.
3) To apply the STL-μ-SPE technique for the determination of PAHs in the selected water and food samples.

1.3 Outlines of the thesis

This thesis is constructed into five chapters. Chapter 1 contains a background of the study, objectives of the research, and the organization of the thesis. The literature review was summarized in Chapter 2 and Chapter 3, discussed the experimental procedures done throughout this project. It is subdivided into five parts, as part one and two all about the chemicals, materials, reagents, and instruments used. While the others about the preparation, applications, method validation, and real sample applications of the proposed method, STL- μ-SPE. Chapter 4 documented the results and discussion of this proposed method. Characterization of STL, optimizations of six parameters, analytical performances, and adsorption mechanism were discussed in Chapter 4. While Chapter 5, is all about the thesis conclusions and future recommendations.

CHAPTER 2 : LITERATURE REVIEW

2.1 Polycyclic aromatic hydrocarbons (PAHs)

2.1.1 Characteristic, sources, and toxicology

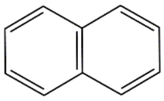
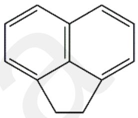
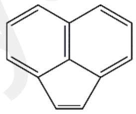
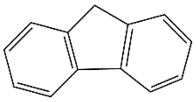
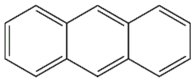
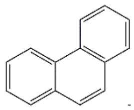
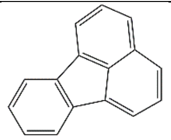
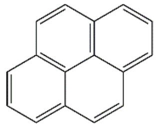
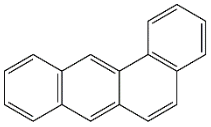
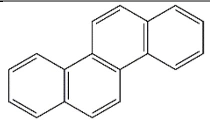
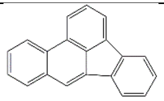
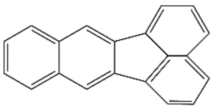
Polycyclic aromatic hydrocarbons (PAHs) are a huge group of persistent organic pollutants that chemically consist of carbon and hydrogen atoms. Two or more benzene rings bonded in a linear, cluster, and angular arrangements (Edward, 1983). Generally, it is very hydrophobic as it detected in very low concentrations in water, specifically 4- and 5- ring compounds. The addition of one aromatic ring can make its concentration in water decrease. It is abundant in the environment and vitally comes from these three sources: pyrogenic, petrogenic, and biological. Pyrogenic PAHs produced when organic material exposed to high temperatures with a short supply of oxygen (e.g incomplete combustion of fuels in car or truck). Usually, it is found in urban areas. While petrogenic PAHs are the product of crude oil maturation. It is dispersed into the environment when there are underwater and above ground storage tank leaks, freshwater oil spill, and a huge release of motor oil and gasoline. Lastly, PAHs also can form biologically. For instance, it is formed during the volcanic eruptions and a product from the degradation of vegetative matter (Abdel-Shafy & Mansour, 2016).

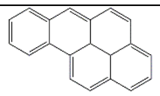
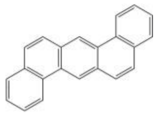
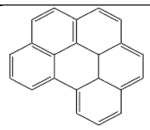
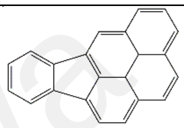
Moreover, PAHs also has been used in a few industries (fluorene used in the production of resinous products and dyes; fluoranthene used in manufacturing agrochemicals, dyes, and pharmaceuticals; pyrene used in pigment industries; chrysene used in organic synthesis; and lastly benzo[a]pyrene used in dyes productions and as laboratory agent (ATSDR, 2020). Their usage in industries results in PAHs emissions into the environment via waste streams and consequently pollutes the environment. Globally, PAHs contamination are happening and might happen on a larger scale, as the population increases, more energy consumptions producing more PAHs into the environment. Proved, there are obvious relations of populations and PAHs global emission by Hafner et al. (2005).

Once released into the environment, PAHs will exist as two separate phases which are the vapor phase and as particulate matter in the solid phase (Caricchia et al., 1999). PAHs classified into two groups by its number of aromatic rings: low molecular weight (LMW) PAHs containing two or three rings and high molecular weight (HMW) PAHs which have more than four aromatic rings (Choudhary & Routh, 2010). Generally, LMW PAHs are more volatile and mainly exist in the gas phase while HMW PAHs exist in the particulate phase as they are insignificant to vaporization (Kameda, 2011). PAHs able to co-exist in solid and gas state, makes PAHs moving freely in the environment and distributed across air, soil, and water bodies (Kim et al., 2013). They are ubiquitous in the environments thus applicable to easily contaminate human beings.

PAHs can affect common populations through breathing contaminated air, digesting food containing PAHs, smoking cigarettes, and inhaling smoke from open burning (Baxter et al., 2014). Short time exposure of high concentration of PAHs to a human being can cause nausea, vomiting, eye irritation and confusion (Unwin et al., 2006), disabled lung function in asthmatics in people who has coronary heart disease (Kim et al., 2013), enhance allergic processes (Schober et al., 2007), and induces inflammatory (Baudouin et al., 2002). The worse long-term effect after exposure to PAHs is jaundice, cataract, kidney, and liver damage (Kim et al., 2013). Other than that, Srogi (2007) stated in contact with naphthalene can cause redness of the skin and inhale or digesting it will break the red blood cells. Besides that, problems may arise upon the exposure of PAHs to the children ‘s respiratory system (Miller et al., 2004). Whereas long-term exposure to PAHs is doubted to surge the risk of cell damage via mutation and cardiopulmonary mortality (Kuo et al., 2003), and exposure to pyrene and benzo(a)pyrene proved to cause cancer in laboratory animals (Diggs et al., 2012). Few epidemiological studies show that mixture of PAHs was carcinogenic especially for lung, skin, and bladder (Armstrong et al., 2004). Hence, without a doubt, PAHs are hazardous to human beings. **Table 2.1** shows all the 16 PAHs with their chemical formula, molecular weight, and molecular structures.

Table 2.1: 16 PAHs listed by EPA as priority pollutant contaminating food and environmental matrices.

Orders	PAHs	Chemical formula	Molecular weight (g mol$^{-1)}$	Structure
1	Napthalene	$C_{10}H_8$	128	
2	Acenapthene	$C_{12}H_{10}$	154	
3	Acenapthylene	$C_{12}H_8$	152	
4	Fluorene	$C_{13}H_{10}$	166	
5	Anthracene	$C_{14}H_8$	178	
6	Phenanthrene	$C_{14}H_{10}$	178	
7	Fluoranthene	$C_{16}H_{10}$	202	
8	Pyrene	$C_{16}H_{10}$	202	
9	Benzo[a]anthracene	$C_{18}H_{12}$	228	
10	Chrysene	$C_{18}H_{12}$	228	
11	Benzo[b]fluoranthene	$C_{20}H_{12}$	229	
12	Benzo[k]fluoranthene	$C_{20}H_{13}$	230	

13	Benzo[a]pyrene	$C_{20}H_{14}$	231	
14	Dibenzo[a,h]antharacene	$C_{20}H_{15}$	232	
15	Benzo[g,h,i]perylene	$C_{20}H_{16}$	233	
16	Indo[123-cd]pyrene	$C_{20}H_{17}$	234	

2.1.2 Occurrences of PAHs in water and food samples

PAHs may enter the water system through atmospheric fallout, municipal effluents, urban run-off, industrial effluents, and oil leakage or spills (Manoli & Samara, 1999). While food contaminated with PAHs may happen over an environmental cause (natural and usually anthropogenic), food processing, and slightly from domestic cooking practices (Zelinkova & Wenzl, 2015). PAHs can get into food matrices through air depositions or by transfer and deposition from soil and water. **Table 2.2** summarizes some type of water and food matrices which analytically proven contaminated by PAHs.

Table 2.2: PAHs detected in water and food sample matrices

Sample matrix	PAHs detected	PAHs concentration	Reference
Cereals, rye bread and wheat bread	BaA, Chr, BbF, and BaP	0.49 to 0.71 $\mu g\ kg^{-1}$	(Rozentale et al., 2017)
Grilled bacon	13 type of PAHs	0.46 to 1.59 $\mu g\ kg^{-1}$	(Ma et al., 2019)
Harbor waters	35 type of PAHs	2.19 to 39.91 $ng\ L^{-1}$	(Dohmann et al., 2019)
Drinking waters	16 types of PAHs	37.93 to 69.81 $ng\ L^{-1}$	(Zhang et al., 2019)
Grilled chicken wings	15 type of PAHs	0.11 to 1.03 $\mu g\ kg^{-1}$	(Ma et al., 2019)
Grilled vegetables	16 type of PAHs	60.4–1936 $ng\ g^{-1}$	(Cheng et al., 2019)
River waters	16 type of PAHs	0.06 to 72.38 $\mu g\ L^{-1}$	(Awe et al., 2020)
Vegetable oil deodorizer distillate	16 type of PAHs	1219.34 to 1482.25 $\mu g\ kg^{-1}$	(Sun & Wu, 2020)
White rice and glutinous rice	Phe and BaA	3.91 to 256.4 $\mu g\ kg^{-1}$	(Hui et al., 2020)
Waste frying oil	16 type of PAHs	39.21 to 197.44 $\mu g\ kg^{-1}$	(Sun & Wu, 2020)

Phe: Phenanthrene, Chr: Chrysene, BaA: Benzo[a]anthracene, BaP: Benzo[a]pyrene, and BbF: Benzo[b]fluoranthene.

Throughout this study, the determination of PAHs was done in four types of matrices: water, rice, orange juice, and apple juice. Rice (*Oryza sativa*) a daily intake food in Asia (Su & Zhu, 2008), if it is contaminated with PAHs, the effect will be critical as the accumulation of PAHs happens in human bodies over a huge rice intake. The contamination of PAHs into rice may happen from the atmosphere, environmental waters, and soil. The deposition of gaseous dry, particulate dry and wet particulate PAHs may happen on the rice plant leaves. Besides that, the root also may adsorb PAHs into the plant through root uptake, translocation, and bio-concentration (Liu & Korenaga, 2001). This route of PAHs contaminations may also happen onto the orange and apple plants, which then eventually bring these hazardous pollutants into the fruits. Moreover, the existence of waxy surfaces on fruits can make low molecular mass PAHs able to accumulate via surface adsorption, and the particulate-bound PAHs stay on the surface through atmospheric fall-out (Ashraf & Salam, 2012). All these sources may stain the rice, orange, and apple fruits with PAHs. Since PAHs are commonly found in food, they can enter the human body easily through ingestion. To prevent this problem from happening, it is crucial to determine PAHs concentration in these foods before it enters the human body.

An attempt to determine these hazardous pollutants in the environment is demanding due to a few obstacles that need to be overcome. First, once released into the environment they can be found in two separate phases [vapor phase and the solid phase where PAHs sorb into particulate matter] (Hyder et al., 2011), PAHs with low vapor pressure (e.g., BaP will be in particulate matter in environment and the one with high vapor pressure like naphthalene will remain in vapor phases), makes the concentration of each type of PAHs is different depending on the type of matrices tested. Second, PAHs usually present in trace levels due to their low water solubility character because they contain lots of aromatic rings. Lastly, hydrophobic PAHs favor to present in hydrophobic matrices, this makes the determination of PAHs in this kind of matrices is challenging as it usually contains lots of matrices interferences. To overcome all these obstacles, a suitable sample preparation technique is needed to identify and quantify PAHs in the environmental and food samples.

2.2 Sample preparation technique

Sample preparation is the preliminary step needed before any analytical determination because the direct determination of trace analyte in complex matrices is difficult due to the deficient sensitivity of the analytical instruments. The isolation and/or enrichment of target analytes happened during this step to make sure the samples were liable to the instrument analysis.

Sample preparation aimed to isolate the target analyte from the sample matrices, to remove the interference or the component that exists in the sample, and to enrich the concentration of the analytes so that it will be above the analytical instrument detection limits before the analysis.

2.2.1 Solid-phase extraction (SPE)

A well-known sorbent-based extraction technique used for the determination of PAHs in environmental samples is solid-phase extraction (SPE). Principally, SPE is about the partitioning of solutes between a liquid phase (sample matrix or solvent containing analytes) and a solid phase. The sorption of analytes from solvent or sample onto solid sorbent allows the pre-concentration and the purification to happen after the extraction (sorbent) (Żwir-Ferenc & Biziuk, 2006). Nowadays, there are varieties of sorptive materials designed and used for the sampling technique. SPE commonly used in chemistry, pharmaceutical, environmental, clinical, food, and industrial chemistry. There are four basic main steps of SPE, (i) conditioning the cartridge sorbent to activate it (pour the solvent into the cartridge), (ii) passing the sample solvent through the cartridge (to allow the sorbent and analyte to have a contact so that the adsorption happens), (iii) washing step to wipe out the impurities (pour the solvent into the cartridge), and lastly (iv) eluting the target analytes by pouring the desorption solvent into the sample vials (pour eluent into the cartridge to detach analytes from the sorbent surfaces). From these steps, large amount of solvent used, tedious extraction procedures, and the needs of abundant sample volume make it less popular.

Then after two decades of rapid evolution in this area of research, different techniques introduced emphasizing miniaturize, simplifying, and automation of extraction mechanism to counter the draw-back of the SPE technique. Eventually, solid-phase micro-extraction (SPME), dispersive solid-phase extraction (d-SPE), and magnetic solid-phase extraction (MSPE) developed and introduced. **Table 2.3**, shows some of the analytical techniques utilized to determine PAHs in a variety of water and food samples. Besides that, porous membrane protected micro-solid-phase extraction (μ-SPE) also developed as an alternative to SPE (Basheer et al., 2006).

Solid phase micro-extraction (SPME) is a process about retaining analytes on the

SPME extraction fibre then the target analyte released through desorption process into chromatography equipment to be detected. SPME known to be lack of robustness (Müller et al., 2006), mechanically weak fibre, poor selectivity, and lastly limited number of commercially prepared fibre coating in market (Spietelun et al., 2010). While technique introduce in this paper is robust as we can use it to extract 5 type of PAHs by using one type of adsorbent and spent tea leaves which used as adsorbent abundant in nature. Other than that, dispersive solid-phase extraction (d-SPE), Eslamizad et al. (2016) applied this process to extract PAHs in bread samples. The main drawback is the sample preparation technique is long and time consuming as they have to make sure other chemical component in the bread did not disturb the PAHs pick up onto their adsorbent surface. By using STL-μ-SPE which introduce through this dissertation, this drawback can be overcome as this method has simpler sample preparations which eventually save times. Third method chosen to overcome LLE and SPE drawback is magnetic solid-phase extraction (MSPE), Boon et al. (2019) utilize this technique for determination of PAHs. The adsorbent preparations step is complex, time consuming, and not cost effective as lot of chemical used. Our newly introduce method is better than MSPE technique for PAHs extraction as the adsorbent preparation is simple, short time, and very cost save.

Table 2.3: Determination of PAHs in water and food samples

Analytical method	Detection	Matrix	LOD	Recovery	References
SPE	GC-MS	Water sample	1.6 – 102 ng L^{-1}	79.1 % to 120.5 %	(Sarria-Villa et al., 2016)
d-SPE	GC-MS	Bread	0.3 – 20.0 ng g^{-1}	97 % to 120 %	(Eslamizad et al., 2016)
d-μ-SPE	GC-FID	Water, vegetables, and fruit juice	1 – 22 ng L^{-1}	97 % - 103.5 %	(Nasrollahpour et al., 2017)
SPME	GC-MS/MS	Honey	0.07 to 12 ng g^{-1}	63 % to 104 %	(Al-Alam et al., 2017)
SPME	GC-MS	Water sample	0.1 – 3.0 ng L^{-1}	82.9 % to 109.2 %	(Zhang et al., 2018)
d-SPE	GC-MS/MS	Edible oil sample	0.06 – 0.21 μg kg^{-1}	96 % to 107 %	(Zacs et al., 2018)
MSPE	GC-FID	Rice sample	0.01 – 0.18 μg kg^{-1}	80.4 % to 112.4 %	(Boon et al., 2019)

SPE: Solid-phase extraction, d-SPE: dispersive-solid phase micro-extraction, SPME: Solid-phase micro-extraction, MSPE: magnetic solid-phase extraction, GC-MS: Gas chromatography-mass spectrometry, GC-MS/MS: Tandem mass spectrometry, and GC-FID: Gas chromatography- flame ionization detector.

2.2.2 Porous membrane protected micro-solid-phase extraction (μ-SPE)

Developed and introduced by Basheer et al, (2006), this method packed a few milligrams of adsorbent inside a porous polymer membrane. The membrane-enclosed by heat seal and then it is known as the μ-SPE device. This device will adsorb target analyte onto sorbent surfaces upon contact with sample solutions, after that in the desorption part, all analyte will detach into eluent which then injected into gas chromatography or liquid chromatography. **Figure 2.1** shows a brief illustration of the extraction steps done for this method. Porous membrane protected μ-SPE is a better version of conventional SPE as it is done in a much smaller scale, simpler procedures, usage of a small number of chemicals, and it also has a high resistance to 'dirty sample', because the adsorbent is protected in the membrane (Basheer et al., 2006). It is also known to be very beneficial because the clean-up and pre-concentration steps happen in single steps (Naing et al., 2016a). In addition, this device can be used more than once, robust, cost, and time-effective micro-extraction technique. Thus, due to these advantages of this μ-SPE technique, it is deployed in this study to determine the hazardous organic pollutant, PAHs.

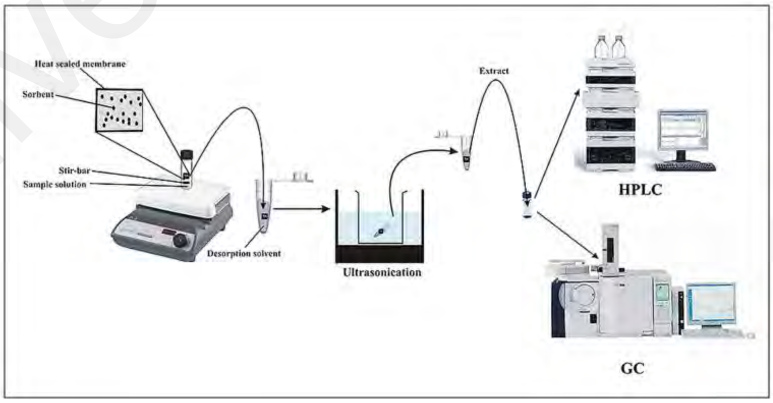


Figure 2.1: The illustration of porous membrane protected μ-SPE (Sajid, 2017).

Numerous researches have done by utilizing this technique, as they varied the type of adsorbent used suitable for the target analyte of studied. Some of them were listed in **Table 2.4**. To the author's knowledge based on the literature review, this analytical technique has never been applied to determine PAHs. Thus, this study will be the first one for this purpose.

Naing et al, (2016a) utilized reduced graphene oxide to extract estrogen as target analyte in water sample and it is detected by HPLC-UV. LOD of this method is between 0.24 and 0.52 ng L^{-1}. Besides, they also utilize chitosan (CS) microsphere as adsorbent with a good detection limit of 0.01 and 0.04 g L^{-1}. But these two processes have a same disadvantage as the adsorbent preparation procedure is tedious and time consuming.

Sajid et al. (2016a) use natural and abundant adsorbent (seed powder of *Moringa oleifera*) to extract phthalate esters in milk sample. The LOD is between 0.01 to 1.2 μg L^{-1}. Our method is based on this study but we change the adsorbent, target analyte, and the sample tested. Besides that, Sajid et al. (2016b) also utilized zinc oxide which combine with carbon form to extract organochlorine pesticides from milk sample with a good limit of detection of 0.19 to 1.64 ng mL^{-1}. But the adsorbent preparations are tedious, long extraction time, and high sample volume needed for each extraction process.

While Lashgari & Lee (2016) extracted perfluorinated carboxylic acids analyte from human plasma sample. This method has a good LOD in range of 21.23 and 65.07 ng L^{-1}. But the sample preparations and adsorbent preparations technique is tedious and time consuming.

Lastly is the molecular imprinted polymer (MIP) adsorbent which used together with this porous membrane protected μ-SPE technique. Adsorbent fabrications take a long time, tedious, and lots of chemicals needed. Thus, increase the cost.

All listed method's drawback is overcome by our newly introduce method STL-μ-SPE, as this method is simple steps to prepare the adsorbent, small sample volume needed, rather short extraction time, and economically low-cost.

Based on **Table 2.4**, the usage of molecular imprinted polymer (MIP) and graphene oxide is favored. However, these adsorbents were tedious to make and rather expensive (Agarry et al., 2013). To reduce the cost and still using the adsorption mechanism to remove persistent pollutants like PAHs, the usage of abundant, available in nature, and eco-friendly adsorbent have attracted the interest of the researchers to replace these adsorbents (Dai et al., 2018). These non-conventional and low-cost adsorbents can be red mud (Çoruh et al., 2011), clay (Errais et al., 2010), plant residue (Chen et al., 2011), and agricultural waste.

Table 2.4: Research done by utilizing porous membrane protected μ-SPE.

Adsorbent used	Target analytes	Matrix	Detector	References
Reduced graphene oxide	Estrogen	Water	HPLC-UV	(Naing et al., 2016a)
Cross-linked Chitosan microsphere	Benzene, toluene, ethylbenzene, xylenes, and styrene	Water	GC-MS	(Naing et al, 2016b)
Seed -powder Moringa oleifera	Phthalate esters (PEs)	Milk	GC-MS	(Sajid et al., 2016a)
Zinc oxide nanoparticles	Organochlorine pesticides	Milk	GC-MS	(Sajid et al., 2016b)
Mesoporous silica	Perfluorinated carboxylic acids (PFCAs)	Human plasma	LC-MS/MS	(Lashgari & Lee, 2016)
Molecular imprinted polymer (MIP)	Cocaine and its metabolites	Plasma sample	HPLC–MS/MS	(Sánchez-González et al., 2016)
Molecular imprinted polymer	Cannabinoids	Plasma and urine sample	HPLC–MS/MS	(Sánchez-González et al.,

(MIP)				2017)
Molecular imprinted polymer (MIP)	Synthetic cathinones	Urine	HPLC–MS/MS	(Sánchez‐González et al., 2019)

GC-MS: Gas chromatography-mass spectroscopy, LC-MS/MS: Liquid chromatography-tandem mass spectrometry, HPLC-MS/MS: high performance liquid chromatography–tandem mass, and HPLC-UV: high performance liquid chromatography- ultraviolet.

2.3 Agricultural waste adsorbent

Agricultural waste potentially can be used as low-cost adsorbents as it represents unused resources, low cost, generally accessible, and environmentally friendly. Besides, the usage of these wastes will decrease the disposal cost and contribute to environmental protection (Olivella et al., 2011). Nowadays, the researchers interested in developing these agricultural wastes as adsorbent of remediation of PAHs as they know that agricultural waste has a high sorption affinity to persistent organic pollutants, abundant in nature, and can be modified to increase its extraction efficiency (Chen et al., 2011).

Crisafully et al. (2008) stated that the adsorption processes governed by the π-π interaction between the sorbent's surface and the PAHs compound. Besides that, Budhwani (2015) also stated that the lignin percentages in the agricultural wastes play major roles in its PAHs absorption ability. Also, the present of cellulose contribute small effect on PAHs adsorption ability (Chen et al., 2011). From these statements, we took up the challenge to find the agricultural waste with (suitable lignin percentage) which wasted every day to be used as an adsorbent to adsorb PAHs.

Table 2.5 shows some of the agricultural wastes utilized as for the removal of PAHs and **Table 2.6** listed the utilization of this waste in the extraction procedure. Variety of agricultural wastes has been utilized as an adsorbent for the removal and extraction of PAHs.

Boving & Zhang (2004) use low-cost aspen wood fibre to remove PAHs and takes 12.5 days for the removal process to reach equilibrium in the laboratory. While Crisafully et al. (2008) found that green coconut shell has the best removal ability than sugar cane bagasse, chitin, and chitosan.

Wood ash wastes incinerated by Pérez-Gregorio et al. (2010) and used as removal adsorbent. They found that higher the carbon content in the adsorbent, the better its

PAHs adsorption's ability. Cock waste also found to be a good PAHs removal adsorbent, with just two minutes exposure to this hazardous pollutant in sample, 80 % of it is already adsorbed on its surfaces (Olivella et al., 2011).

Other than that, soybean stalk-based carbon is prepared by Kong et al. (2011) for removal of PAHs, they found that this adsorbent removal ability is better than the commercial activated carbon. Besides, Ngo et al. (2015) listed the character of lignocellulose which able it to adsorb organic pollutant: its functional group, surface morphology, porosity, surface area, and its chemical composition. Lastly, De Jesus et al. (2017) coconut waste and used orange as removal adsorbent of PAHs. They found that, the removal percentage is 30.33–83.43% and 24.20–74.25%, respectively.

But all method listed in Table 2.5 is only for removal procedure, they cannot do extraction procedure. But these indicates that agricultural waste can be used as an adsorbent. As seen in **Table 2.6**, there are few kinds of researches done utilizing agricultural adsorbent for the determinations of PAHs.

Wang et al. (2014a) embedded alkylbenzenesulfonates onto egg shell membrane and use it to extract PAHs in environmental sample. The LOD of this method is 0.1 – 8.6 ng L^{-1}. The main drawback is the egg shell membrane is a complex biomaterial which contain lots of possible impurities, long steps of washing and rinsing with few chemicals is needed. This is time consuming and not economical different from the introduced method in this project, which is simple and low cost.

Besides, natural cotton fibre also utilized as PAHs adsorbent without any chemical modifications with good LOD of 0.1 – 2.0 ng L^{-1} and 70.69 % to 110.4 % recovery percentage (Wang et al., 2014b). Though it is low-cost and simple, the extraction time is too long as they need 1 hour of stirring and also need 2.0 mL of eluent for the elution process. While our method just needs 12 minutes for extraction time and 0.5 mL of eluent.

Third method listed on Table 2.6 was utilizing eggshell with graphene quantum dots nanocomposites, this nanocomposite is synthesized by immersing the fresh eggshell into graphene quantum dots solution. The LOD and recovery of this method is 5 – 75 ng L^{-1} and 92.4 % - 113.8 % (Razmi et al., 2016), respectively. This method needs 200 mg of the modified eggshell membrane, thus lots of eggs needed for the validations and optimization steps. It is tedious and time consuming different from STL-μ-SPE which just utilized natural abundant STL as an adsorbent.

Forth method listed is done by Singh et al. (2020), they gone through a lots of adsorbent preparations procedure to extract silica from rice husk and then modified it to become silica nanoparticles before it is used as an adsorbent of PAHs in wastewater sample. The LOD and recovery percentage is 1.5 x 10-6 – 1.0 mg L^{-1} and 94.7 % to 99.9 %. The tedious adsorbent's preparations technique makes this method less favorable compared to our method did not do any chemical modifications on the adsorbent surfaces. Less chemical used, safer, and obviously less time consuming.

Lastly, by utilizing activated carbon based from grape leaf litter, Awe (2019) able to determine PAHs in water and sediments samples with LOD of 0.02 – 0.04 μg mL^{-1} and percent recovery of 70.35 % to 100.83 %. The disadvantage of this method is the adsorbent's preparations step where they need a high heat to transform leaf litter to activated carbon, where time and special furnace is needed. Our method has a better adsorbent's preparations technique as it is simple and did not need special equipment to fabricate.

Due to these reasons, we introduce our method which utilizing other type of adsorbent with simpler preparation procedure and low-cost.

Table 2.5: The application of agricultural waste for the removal of PAHs.

Waste adsorbent	Matrix	References
Aspen wood fibre	Water sample	(Boving & Zhang, 2004)
Green coconut shell	Petrochemical wastewater	(Crisafully et al., 2008)
Wood ash wastes	Organic solvent	(Pérez-Gregorio et al., 2010)
Quercus cerris cork	Aqueous solution	(Olivella et al., 2011)
Soybean stalk based activated carbon	Water sample	(Kong et al., 2011)
Sugar cane bagasse	Water	(Ngo et al., 2015)
Coconut shell based activated carbon	Sea water	(Amstaetter et al., 2012)
Unripe orange peel	Aqueous solution	(Owabor et al., 2012)
Rice straw	Petroleum refinery water	(Younis et al., 2015)
Banana peel activated carbon	Aqueous solution	(Gupta & Gupta, 2016)
Orange waste	Sea and river water	(De Jesus et al., 2017)
Coconut waste	Sea and river water	(De Jesus et al., 2017)

Table 2.6: The applications of agricultural waste for the extraction of PAHs.

Waste adsorbent	Detection method	Matrix	LOD	Recovery	References
Modified egg shell membrane	SPE-HPLC-UV	Environmental aqueous sample	0.1 – 8.6 ng L^{-1}	77.8 % to 112.7 %	(Wang et al., 2014a)
Natural cotton fibre	SPE-HPLC	Water samples	0.1 – 2.0 ng L^{-1}	70.69 % to 110.4 %	(Wang et al., 2014b)
Graphene quantum dots–eggshell nanocomposite	SPE-HPLC	Water samples	5 – 75 ng L^{-1}	92.4 % - 113.8 %	(Razmi et al., 2016)
Silica nano-powder extracted from rice husk	SPE-GC-MS	Wastewater sample	1.5 x 10^{-6} – 1.0 mg L^{-1}	94.7 % to 99.9 %	(Singh et al., 2020)
Activated carbons from Vitis vinifera (grape) leaf litter	SPE-GC-FID	Water and sediments samples	0.02 – 0.04 µg mL^{-1}	70.35 % to 100.83 %	(Awe, 2019)

GC-MS: Gas chromatography-mass spectroscopy, HPLC: high performance liquid chromatography, HPLC-UV: high performance liquid chromatography- ultraviolet, and GC-FID: Gas chromatography- flame ionization detector.

2.3.1 Spent Tea leaves (STL)

Camellia sinensis leaves are dried and processed to produce tea (Hameed, 2009). As a second most consumed beverage worldwide after water, the world tea production including all types of tea rises by 4.4 % per year last decade to reach 5.3 million tonnes in 2016 (Utkina, 2018). Approximately, 18 to 20 billion cups of tea are drunk every day in the world (Hussain et al., 2018). To abide by the needs, the production of tea increases, and eventually the tea waste also increased, the safe disposal of tea leaves becomes a huge concern. The improper disposal of tea waste will result in environmental problems. In Malaysia alone, the factory-rejected tea per annum is around 100,000 tons. While in Turkey, reported about 30,000 tons of tea waste from the factory is disposed of in the small bays around the Black Sea (Yagmur et al., 2008).

Known that tea waste's physiochemical character (huge surface capacity and fast kinetics of adsorptions) is suitable to be used as adsorbent (Hussain et al., 2018). Knowing this fact, the researcher is trying to utilize this waste by using it as an adsorbent, summarized in **Table 2.7**. This will somehow lessen the landfill needed for the dumping of spent tea leaves (STL) and also make it useful for another purpose by removing hazardous pollutants from the environment.

STL produced after the tea leaves were brewed for the consumption. Black tea has gone through a complete fermentation process (Sivakumaran & Amarakoon, 2017) then it is brewed, dried, and ground before applied as an adsorbent. Black tea leaves consist of cell wall materials, hot water dissolved polysaccharides and protein together with the lignin, a structural protein, cellulose, and protein which insoluble in hot water (Tee at al., 1988). The components in the insoluble cell wall are lignin, cellulose, hemicellulose, and the condensed tannins (Thapak et al., 2015). These components do contribute to the STL's adsorption ability: lignin proven to be one of the contributors by Crisafully et al. (2008) and Budhwani (2015), cellulose and hemicellulose do slightly contribute to the uptake of PAHs (Huang et al, 2006). In addition, the presence of 80.1 mgg polyphenols (Yashin et al., 2015) in it does affects the PAHs adsorption ability. These groups have lots of aromatic rings that may have hydrophobic interaction with the aromatic ring of PAHs, thus facilitate the adsorption of PAHs onto tea surfaces.

Other than that, the aliphatic carbon present in STL plays a major role on its PAHs adsorption ability. If STL is properly managed, it can act as an excellent adsorbent to clean up the contaminated water (Lin et al., 2007).

Table 2.7 shows the applications of STL as adsorbent. In general, STL used as a removal adsorbent of dyes and metal ions. Lazim et al. (2015) washed STL with tap water and distilled water few times before dried it in oven for 24 hours and sieve it through 30 mesh, it is used as removal adsorbent of Remazol Brilliant Blue R dyes. The removal percentage after 24 hours are 11.39 %. The adsorption happened due to the presence of lignin, cellulose, hemicellulose, pectine, low molecular weight hydrocarbons, and also the hydroxyl group such as carboxyl and hydroxyl as these functional group helps to attract analyte from the sample solution (Lazim et al., 2015).

In other work, Heraldy et al. (2016) applied STL for removal of Procion red MX 8B dye, with adsorption capacity of 3.28 mg/g. The STL adsorbent is activated by 4 % Sodium Hydroxide before usage. They found that lignin in STL contributed in the adsorption mechanism where chemical interactions happened between adsorbent surface and the analyte (Heraldy et al., 2016).

Besides, Crystal Violet dye also adsorbed by STL, with adsorption capacity of 175.4 mg/g and they found that STL can be used to remove basic dyes in industrial wastewater treatment (Khan et al., 2016).

In 2018, Khan et al. (2018a) successfully removed Eriochrome Black T (EBT) dye by using microwave-assisted spent black tea leaves (MASTL) sorbent. The modification process helps to increase the surface area of STL adsorbent, result shows this method's adsorption capacity is 242.72 mg/g. The adsorption happened at pH 2.0, between positive charged adsorbent's surface interacting with the negatively charged EBT analyte molecules (Khan et al., 2018a).

Liu et al. (2018) adsorbed Methylene Blue dyes from aqueous solution with adsorption capacity of 113.1461 mg/g and they concluded that organic functional group in STL majorly contributed on its adsorption ability.

Khan et al. (2018b) concluded that STL capable to remove Congo Red dyes with more than 80 % removal percentage with 100 mg STL dosage. Their STL adsorbent is washed with boiling water repeatedly, dried in oven for 24 hours, and sizes reduced by using pestle and mortar before it is ready to be used as removal adsorbent.

Wong et al. (2018) utilized STL based activated carbon for removal of aspirin in aqueous solution. STL firstly prepared same as this dissertation then it is immersed in activating agent before it is carbonized at 600 °C for 1 h in an oven. They found that the at optimum condition this method able to remove aspirin at 94.28 % after 60 min of contact time. The interaction between STL-based activated carbon and aspirin most likely attributed by the adsorbent surface, pore properties, and the interactions of oxygenated groups on adsorbent surface with the analyte molecules.

While Ali et al. (2018) used activated carbon from STL to remove phenol by using ultrasound assisted adsorption process. They found that this type of activated carbon derived from STL has a great surface area and the maximum adsorption percentage is 85 % in 60 minutes. The removal of phenol happened as the phenol degraded into catechol (Ali et al., 2018).

Chromium (IV) metal ions successfully removed by using STL adsorbent from leather tanning wastewater (Nur-E-Alam et al., 2018). The adsorption capacity is 10.64 mg/g. They concluded that STL is low-cost, abundantly available, and an efficient bio-adsorbent for this application. The metal ion uptake happened due to the presence of, hemicelluloses, lignin, cellulose, condensed tannins, and structural proteins in the STL (Nur-E-Alam et al., 2018).

STL also modified with polyethyleneimine and applied as removal adsorbent of Reactive Black 5 (RB5) and Methyl Orange (MO) and the adsorption capacity is 1.9 mg/g and 62.11 mg/g, respectively. They concluded that functional group modifications on bio-sorbent with a correct extraction method can save cost and less complicated than preparing an activated carbon. They found that chemical interaction happened responsible for the adsorption process: the PEI groups attached to the STL with the surface charged ends of the dye anions (Wong et al., 2019).

Tesfagiorgis et al. (2020) removed Cobalt (II) ion by utilizing STL in fixed bed column experiments three type of tea used: green tea, peppermint tea, and chamomile tea. They found that peppermint tea is the best among three for the removal of Cobalt (II) ions. Adsorption capacity of peppermint tea, green tea, and chamomile tea were 59.7, 25.2, and 24.9 mg/g, respectively. Lignocellulosic materials like hydroxyl, amino, carboxyl, sulfonic, thiol, hemiacetal, and imine in STL contributed in the Cobalt (II) ion removal.

A simple modification of tea waste with magnetic iron oxide nanocomposite prepared and utilized as removal adsorbent of Lead (II) ion from aqueous solutions. The adsorption capacity of this method is 18.83 mg g^{-1}. They found that their method's main advantage is easy and simple magnetic separation. They found that the great surface area on their STL adsorbent, increase the number of surface hydroxyl groups, a major contributor for the rapid Lead (II) ion removal.

However, all method mentioned above were only for removal purpose. The extraction and detection have not been done before, to the best of author knowledge.The application of STL on PAHs as an extraction adsorbent in sample preparations has not reported yet. Thus, in this study is the first study done by utilizing STL as an adsorbent for the determinations of PAHs.

Table 2.7: The applications of spent tea leaves (STL) as an adsorbent for removal procedure

Analyte	References
Remazol Brilliant Blue R dyes	(Lazim et al., 2015)
Procion red MX 8B	(Heraldy et al., 2016)
Crystal violet dye	(Khan et al., 2016)
Eriochrome Black T (EBT) dye	(Khan et al., 2018a)
Methylene blue (MB)	(Liu et al., 2018)
Congo red dyes	(Khan et al., 2018b)
Rhodamine B dyes	(Mitra, & Mukherjee, 2018)
Aspirin	(Wong et al., 2018)
Phenol	(Ali et al., 2018)
Chromium (VI)	(Nur-E-Alam et al., 2018)
Penicillin G	(Silva et al., 2019)
Reactive Black 5 (RB5) and Methyl Orange (MO)	(Wong et al., 2019)
Cobalt (II) ion	(Tesfagiorgis et al., 2020)
Lead (II)	(Khanna et al.,2020)

CHAPTER 3 : METHODOLOGY

3.1 Chemicals, materials, and reagents

Black tea leaves purchased from supermarkets in Pantai Dalam, Kuala Lumpur. Teabag [non-woven fabrics made of Polyethylene (PE) and Polypropylene (PP)] bought from Daiso, Japan was used as a porous membrane in this study. Hexane, acetonitrile, toluene, methanol, and ethyl acetate were purchased from Merck (Darmstadt, Germany). The standard references of PAHs: fluorene (Flu), fluoranthene (Flt), pyrene (Pyr), chrysene (Chr), and benzo(a)pyrene (BaP) purchased from Supelco (Bellefonte, USA). PAHs stock solutions prepared in methanol at a concentration of 100 mg L^{-1} and stored in a dark amber glass at 4 ºC until further use. The standard solutions were freshly prepared with ultrapure water.

3.2 Instruments

Five types of PAHs were separated and quantified using the Agilent 7890A GC system with Agilent 5975C Series GC-FID from Agilent Technologies Inc. (Santa Clara, CA, USA). Injector and detector temperature were 300 ºC and 330 ºC, respectively with spiltless mode. The separation was carried out by using HP5-MS fused silica capillary (5 %-Phenyl)-methylsilaxone with 30 m x 25 mm I.D, and 0.25 µm stationary film thickness. The carrier gas was at a constant flow rate of 30 mL min^{-1} of N_2. The GC oven was set as follows: 90ºC (hold 5 minutes) and ramped to 290 ºC at 10 ºC/min and held for 1 minute. The overall analysis time is 26 minutes. The functional group in the adsorbent was characterized by using the FT-IR spectrometer (Spectrum 400 Perkin Elmer, Waltham, MA, USA) with a diamond ATR accessory, using absorption mode with 4 scans at a resolution of ± 4 cm^{-1}, and a wavenumber range 4000 to 450 cm^{-1}. The surface morphology and the elemental analysis of STL adsorbent were investigated by using field emission scanning electronics microscopy (FESEM HITACHI SU8220, OXFORD Instruments) equipped with energy dispersive X-ray spectrometry (Carl-Zeiss, Germany).

3.3 Preparations of adsorbent

3.3.1 Preparation of spent tea leaves

The spent tea leaves (STL) prepared by boiling the processed tea leaves bought from the supermarket with distilled water repeatedly until a colorless filtrate observed, this is done to remove any color and soluble components in the tea leaves. After that, spent tea leaves (STL) were dried overnight in an oven at 80 ºC which then ground and screened through a 65-mesh sieve before stored in a tight and amber bottle until further use.

3.3.2 Fabrication of µ-SPE device

The µ-SPE device prepared by enclosed the STL in the PP and PE teabag filter. The commercially bought tea bag filter was folded and cut as shown in **Figure 3.1**. Firstly, a two-layered square shape of 1.5 cm x 1.5 cm was cut, folded, and two of its edge was heat sealed. The measurement is made to 1.5 cm x 1.5 cm to give extra space for the other two ends to be sealed. Two-layered of the porous membrane were designed to make sure the STL did not leech out from the device. Then after STL weighed, it is inserted into the envelope through the remaining open end and then cut and sealed to make a 1 cm x 1 cm square envelope. Each µ-SPE device then cleaned and conditioned by dipping it into acetone and sonication for 10 minutes before it is dried and kept in a tight bottle until further use. Each porous membrane took 2 minutes to be prepared and 30 devices can be made in an hour. This is the advantage of this method as the non-tedious preparations step makes it better than the conventional SPE.

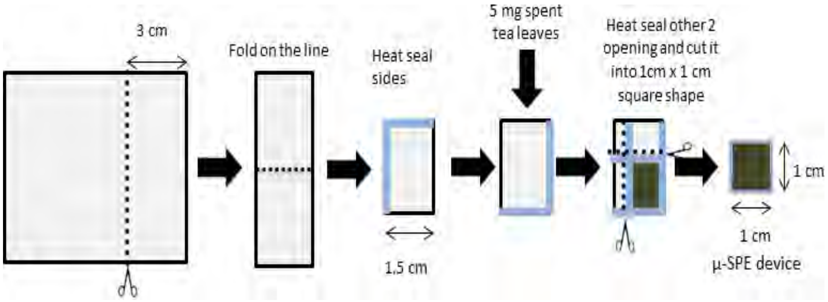


Figure 3.1: The fabrication of µ-SPE device

3.4 The application of STL as adsorbent

The STL-µ-SPE study done as in **Figure 3.2**, 5 mg of STL enclosed in porous membrane added into 30 mL glass vials containing 5 mL of the spiked sample solution. Adsorption procedure is done by vials sonication for 12 minutes, after that the µ-SPE device took out using tweezers from the vials and dried on lint-free tissue for 5 minutes. The desorption process continued by adding 500 µL of desorption solvent into 3 mL vials containing the dried µ-SPE device, then sonication for 10 minutes. After that, the eluent decants into small vials before 1 µL of it injected into GC-FID.

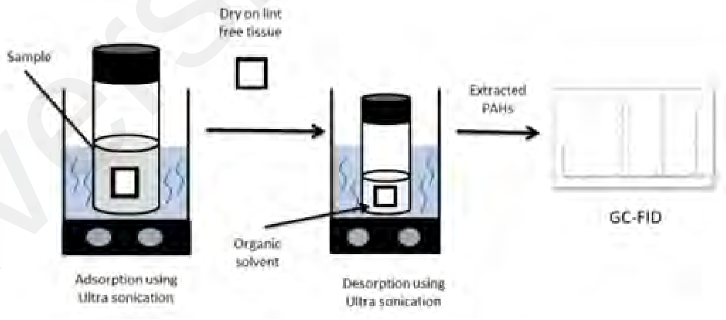


Figure 3.2: The illustration of micro-SPE procedure

3.5 Optimization of STL-µ-SPE method

In order to get an optimum extraction performance, few parameters were studied: adsorbent dosage, time of adsorption, time of desorption, type of eluent, the volume of eluent, and the sample volume. These parameters analyzed in water samples spiked with 5 mg/L of five types of PAH (Flu, Flt, Pyr, Chr, and BaP) and all analyses done in triplicate (n=3).

3.5.1 Optimization of type of eluent

A solvent that can efficiently detach the PAHs from the STL surfaces is needed. In order to do this, four types of solvent used: acetonitrile, hexane, ethyl acetate, and toluene. The optimization of this parameter done by setting the other parameter as constant: the dosage of sorbent, 10 mg; adsorption and desorption time, 10 minutes; the volume of eluent, 500 µL; the volume of sample, 5 mL.

3.5.2 Optimization of adsorbent dosage

The optimum dosage of STL sorbent was investigated by varying the amount of STL packed into the µ-SPE device. It was varied as 5 mg, 15 mg, 25 mg, and 50 mg. The other parameters were kept constant while doing this optimization: eluent, hexane; adsorption and desorption time, 10 minutes; the volume of eluent, 500 µL; the volume of sample, 5 mL.

3.5.3 Optimization of the adsorption time

The time given for the interaction between PAHs compound and STL surface is called the adsorption time. In this phase, PAHs analytes will adsorb on the STL surfaces. The more time given the more attachment happens, but if it is too long the detachment may also happen as the analyte dissolve back into the solution. Thus, it is crucial to investigate this parameter and it is done by varying the adsorption time in a series of 2, 5, 10, 12, and 15 minutes while keeping other parameters constant: eluent, hexane; the adsorbent dosage, 5 mg; the desorption time, 10 minutes; the volume of eluent, 500 µL; the volume of sample, 5 mL.

3.5.4 Optimization of the desorption time

This parameter allows the detachment of PAHs from the STL surfaces into the organic solvent. If the time too short, only a small number of PAHs detached while some of it will still attach on the adsorbent's surface. But if the time of desorption is too long, PAHs might re-adsorbed on the STL's active sites again. Hence, to know the optimum desorption time, the desorption time was varied as 2, 5, 10, and 12 minutes while keeping others parameter constant: eluent, hexane; adsorbent dosage, 5 mg; the adsorption time, 12 minutes; the volume of eluent, 500 µL; the volume of sample, 5 mL.

3.5.5 Optimization of the volume of eluent

An ideal volume of eluent is necessary, if the volume is too high the target analyte detected by GC-FID will be too small as the analyte is diluted too much in the huge volume of an eluent. While if the eluent volume is too small, the µ-SPE device will be not fully in contact with the eluent, thus decrease the detachment of PAHs from the STL surfaces. In order to find the optimum eluent volume, the optimization was done by varying the eluent volume: 500, 750, 1000, and 2000 µL while keeping other parameters constant: eluent, hexane; adsorbent dosage, 5 mg; the adsorption time, 12 minutes; desorption time, 10 minutes; the volume of sample, 5 mL.

3.5.6 Optimization of sample volume

The sample volume was optimized by varying it in a series of 1, 3, 5, 10, and 20 mL. This parameter is directly related to the adsorbent's loading capacity, if the sample (containing PAHs) volume is more than the adsorbent's loading capacity, the excess sample volume will just become a waste. While if it is too small, then the adsorbent's active sited is not fully loaded and disrupt the extraction efficiency. Thus, the optimization of this parameter is crucial and done by keeping other parameters constant: eluent, hexane; adsorbent dosage, 5 mg; the adsorption time, 12 minutes; desorption time, 10 minutes; the volume of eluent, 500 µL.

3.6 Reusability and carryover study

Adsorption and desorption experiment was done for seven times consecutively on the same µ-SPE device, to learn this method ‘s reusability and carryover. After the first desorption step, the device is air-dried and then another 500 µL of hexane added into desorption vials and sonication done for 10 minutes. The eluent injected into GC-FID, to study the carryover of any target analyte on STL surfaces. While the reusability study was done by repeatedly did the extraction procedure on the µ-SPE device (each cycle, the device rinsed with hexane).

3.7 Method validation

3.7.1 Linearity and precision

Four matrices matched the calibration curves were constructed under the optimum extraction condition. Series of spiked samples in the range of 50 and 1000 ng mL^{-1} in deionized water, orange juice, and apple juice while in the range of 50 and 1000 µg kg^{-1} in rice were constructed to make a calibration curve (n=3). Methods precision was determined by calculating the intra-day and the inter-day variation of extraction result. Intra-day or repeatability measured by injecting six replicates of extraction in the same days while the inter-day or intermediate determined by six consecutive days of the extraction procedure. A precision study was done in a deionized water sample at a spiking level of 700 ng mL^{-1} and it is expressed as relative standard deviation (RSD %), using **Equation 3.1:**

$$\boldsymbol{RSD = \frac{SD}{\overline{x}} \times 100}\ \% \qquad \textbf{Equation 3.1}$$

where $\overline{x}$ is mean value and SD is the standard deviation for the sample analyzed.

3.7.2 Limit of detection (LOD) and limit of quantification (LOQ)

LOD does define the lowest concentration of target analyte that is detectable but not necessarily quantified. While LOQ defined as the lowest concentrations of analyte that can be quantified with good precision and under optimum condition. These two parameters determined from SD of blank response ($\boldsymbol{\sigma}$) and its slope (S) from the calibration curve prepared, using **Equation 3.2** and **Equation 3.3**:

$$LOD = \frac{3.3 \times \sigma}{S} \qquad \textbf{Equation 3.2}$$

$$LOD = \frac{10 \times \sigma}{S}$$ **Equation 3.3**

Where σ is the standard deviation of blank residual of injections while S is the gradient of the linear calibration curve (Sanagi et al., 2009).

3.7.3 Pre-concentration factor (PF)

The pre-concentration factor is the ratio of analyte in the enrich phase after extraction and the initial concentration of an analyte in the aqueous sample (Mazloomifar, 2013). This factor does symbolize the ability of an adsorbent to enhance the analytical signal of target analyte after the extraction process. The higher the PF, illustrate the better performance of the adsorbent. PF is calculated based on the extraction of PAHs at spiking level of 700 ng mL^{-1} in water, orange juice, apple juice, and 700 μg kg^{-1} in rice sample, based on **Equation 3.4** (Kamaruzaman et al., 2013; Mukhtar & See, 2016):

$$PF = \frac{final\ concentration\ in\ enrich\ extract}{initial\ concentration\ in\ sample\ solution} \times \frac{extraction\ volume}{desorption\ volume}$$ **Equation 3.4**

3.7.4 Matrix effect

Matrix effect has a high impact on linearity, reproducibility, and accuracy of method on quantifying the target analyte in a sample due to the co-eluting matrix which caused either enhancement or suppression on analytes signal (Cappiello et al., 2008). In order to approximate matrix effect, happen in each sample matrices, the slope of matrix-matched calibration curved which prepared for each matrix was compared with the slope from the spiked water sample by assuming no matrix effect happens in the water sample. The matrix effect expressed in terms of percentage (%) (Hui et al., 2020). If the percentage of difference is not too high, it can be concluded there are no obvious enhancement or suppression happen due to the co-eluting matrix.

3.7.5 Relative recovery study

To access the ability of the STL-µ-SPE method to recover the target analytes in real samples, a mixture of 5 PAHs injected into real samples at two different concentration levels: 700 and 400 ng mL^{-1} in water, orange and apple juice, while 700 and 400 µg kg^{-1} in rice sample. Recovery result expressed in the percentage of analyte detected in the spiked sample (n=3) versus the peak area of PAHs on the calibration curve. The higher the recovery percentage, the better the performance of an adsorbent to recover the analytes in the real sample, which is challenging as there are other components that exist in the real sample that may disrupt the adsorbent performance.

3.8 Real sample analysis

36 different samples of water, orange juice, apple juice, and rice were tested by using this method in order to quantify PAHs in these samples. 9 water samples included: 7 rivers, one well, and one tap water sample. River and well water samples collected around Melaka, Malaysia while tap water is taken from our lab (University of Malaya, Kuala Lumpur, Malaysia). All water samples filtered through 0.45 µm Nylon filter and then stored at –4 ºC until analysis. 18 orange and apple fruits samples which bought from the different supermarkets (Kuala Lumpur, Malaysia). The fruit was cut into small pieces, homogenized using a laboratory homogenizer, and then treated like Zhao et al. (2009) with slight modifications. The homogenized juice then centrifuged for 10 minutes at 3,000 rpm, the supernatant decanted into beaker before it is filtered through 0.45 µm nylon filter and till further use, all juices were stored in dark at -4 ºC. Lastly, the rice samples were treated like Boon et al. (2019) with small changes. 9 different types of rice samples purchased from supermarkets (Melaka, Malaysia). Rice bought were homogenized, filtered through a commercial strainer, and kept in glass container before 0.5 g of it is were spiked with PAHs mixture and then treated with 0.6 mL of acetonitrile in 15 mL centrifuge tube. After that, the mixture was centrifuge for 10

minutes at 3,000 rpm. Then the supernatant decanted into a volumetric flask and deionized water added to make 5 mL of sample volume. STL-μ-SPE procedure done on these samples and then the eluent injected into GC-FID for the analysis.

CHAPTER 4 : RESULT AND DISCUSSION

4.1 Characterization of STL adsorbent

4.1.1 FTIR analysis

FTIR analysis was conducted to characterize STL, to investigate the functional group present in it. **Figure 4.1** shows the FTIR spectra of STL.

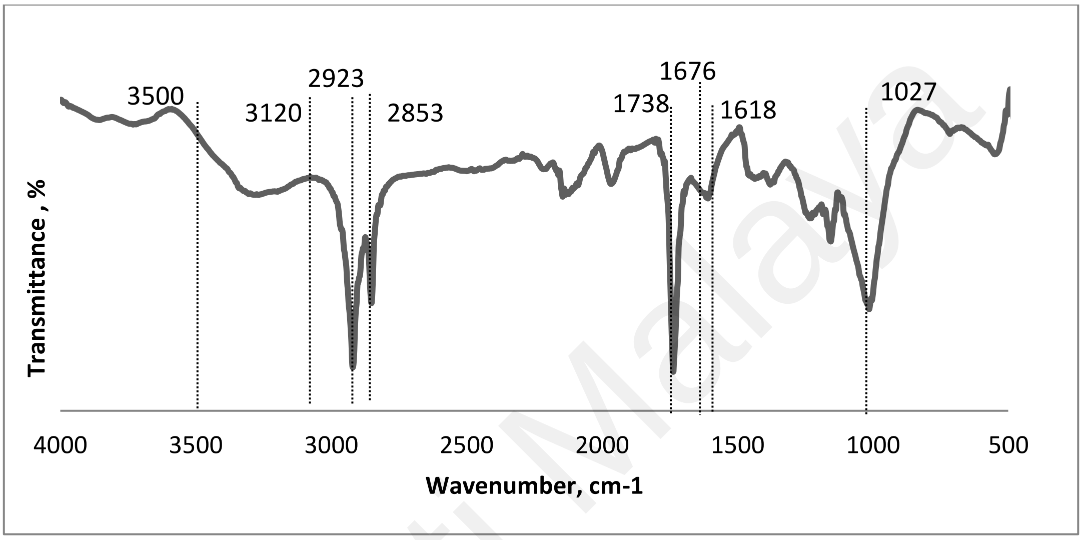


Figure 4.1: The FT-IR spectra of STL

The FT-IR spectra between 4000 and 450 cm^{-1} for the STL are shown in **Figure 4.1**. Based on the adsorption band we can identify the functional group present in STL. Broad adsorption around 3500 cm^{-1} and 3200 cm^{-1} indicated the presence of the hydroxyl group (-OH). This signal might come from H_2O (3500 – 3200 cm^{-1}), alcohols and phenols (3650-3200 cm^{-1}), or -COOH (3400 – 2400 cm^{-1}) (Kang & Xing, 2005). Besides, an aliphatic carbon present as there are double peaks at 2923 and 2853 cm^{-1} adsorption band. Carbonyl peak (C=O) also observed at 1738 cm^{-1}, it may belong to the carboxylic group and together with 1027 cm^{-1} peak which belongs to the C-O stretching of polysaccharides, indicating STL does contain abundant of carboxylic acid in it (Chefetz, 2003). While peak at 1676 cm^{-1} indicates the amide C=O stretch and peak 1618 cm^{-1} assigned as skeletal vibrations of C=C stretching vibration (aromatic rings) (Lin et al., 2007).

4.1.2 Field Emission Scanning Electron Microscope analysis (FESEM)

FESEM analysis done to investigates the morphology of STL adsorbent. FESEM image shown in **Figure 4.2**:

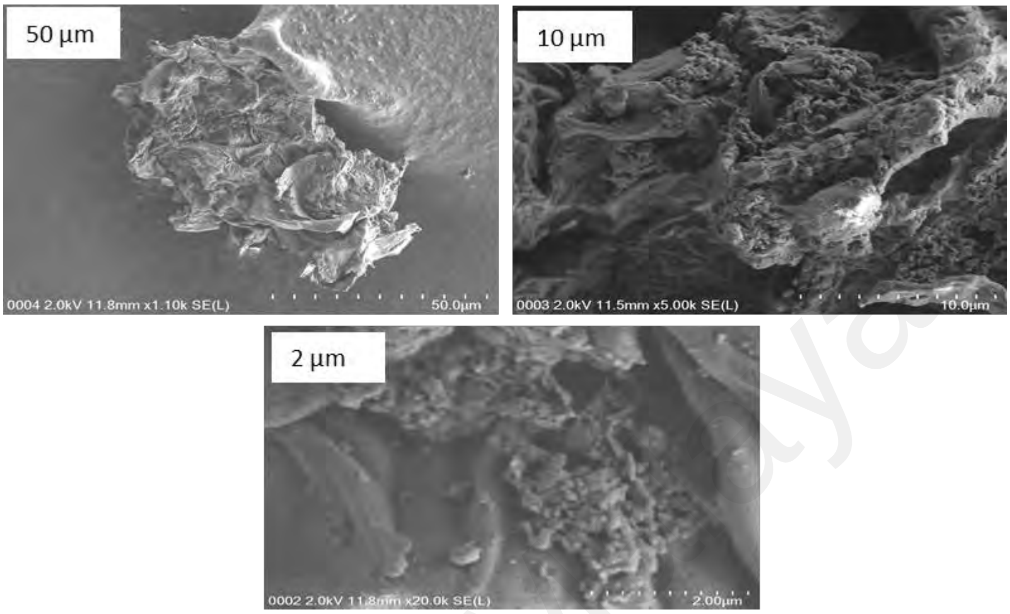
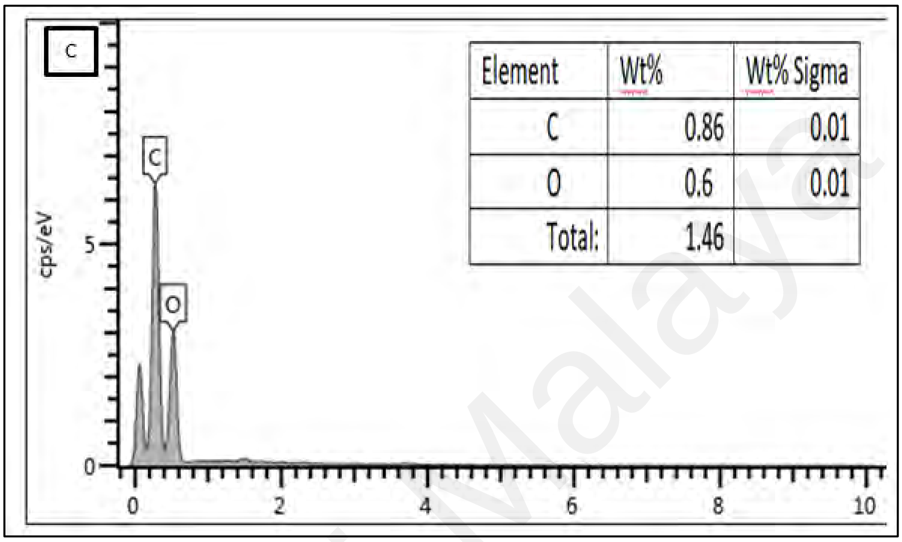


Figure 4.2: FESEM images of STL

From images in **Figure 4.2**, the STL has a heterogeneous surface because there were hilly and valley-like structures on its surface. Lazim et al. (2015) stated that this type of surface is ideal as the adsorption sites of pollutants. Besides that, STL 's uneven surface does increase the surface area exposed to the analyte. Thus, help to increase the extraction efficiency.

4.1.3 Energy-dispersive X-ray spectroscopy (EDX)

EDX was done to investigate the element present in STL. EDX result shows in **Figure 4.3**: The result clearly shows that only carbon and oxygen elements detected in STL. This analysis result agrees with the FTIR analysis result as STL mainly consists of –OH, - CH_2, and C=O.



Element	Wt%	Wt% Sigma
C	0.86	0.01
O	0.6	0.01
Total:	1.46	

Figure 4.3: EDX analysis result of STL

4.2 Optimization of STL-μ-SPE technique

After the morphology and chemical content of STL was confirmed, it is applied as an adsorbent for the determination of five types of PAHs (Flu, Flt, Pyr, Chr, and BaP) in selected food and environmental samples. To have an optimum extraction condition, the optimization step was done on few extraction parameters: type of eluent, the dosage of sorbent, adsorption time, desorption time, the volume of eluent, and volume of sample.

4.2.1 Type of eluent

Types of eluent play an important role in the desorption of analytes from the adsorbent 's surface. In order to choose the suitable eluent to be used, four types of solvent: acetonitrile, hexane, ethyl acetate, and toluene utilized as the desorption solvent. For each solvent tested, 500 μL of it were used in th e STL-μ-SPE procedure. The choice of eluent depends on its ability to dissolve the target analyte (Galán-Cano et al., 2013). As the number of benzene rings fused in PAHs increases, its hydrophobicity will increase (Juhasz & Naidu, 2000) and determining the type of solvent suitable to elute it. Form all four eluents tested, hexane (the most hydrophobic solvent out of four solvent used) able to detach PAHs from the STL surface efficiently. This agrees with the fact that the hydrophobic PAHs need a hydrophobic solvent to detach it from the adsorbent surfaces, and the more hydrophobic the solvent the better its performances. This observation also agrees with the principle of 'like dissolve like' (Ge & Lee, 2011), where hydrophobic PAHs favor more hydrophobic solvent. **Figure 4.4** shows hexane is the best eluent to be used in this proposed method. Thus, hexane selected as eluent throughout the experimental procedure.

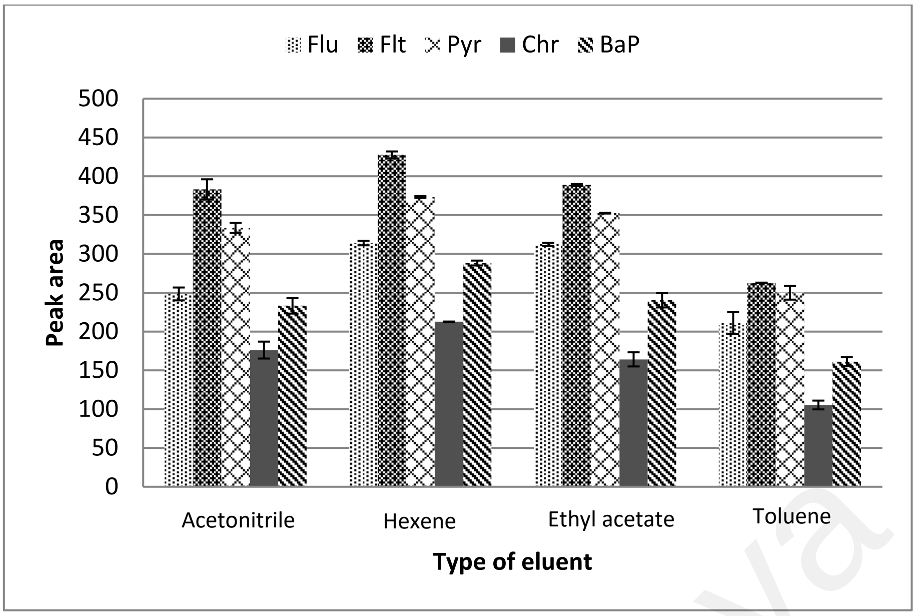


Figure 4.4: The effect of eluent type on the extraction efficiency. (Extraction conditions: The dosage of sorbent: 10mg; Adsorption and Desorption Time: 10 minutes; The volume of eluent: 500 µL; The volume of sample: 5 mL)

4.2.2 Adsorbent dosage

To investigate the relationship of sorbent quantity and its ability to extract PAHs, the dosage of STL packed in the µ-SPE device was optimized. Thus, the dosage was varied in the range of 5.0 to 50.0 mg. By referring to **Figure 4.5**, the extraction efficiency decreases with the increment of adsorbent dosage. Hence, 5 mg of STL picked as an optimum dosage of adsorbent throughout the project. This phenomenon can be explained since any higher dosage of sorbent added in constant sample volume and contact time had decreased the efficiency of the elution process (Boon et al., 2019). Other than that, particle aggregation happened among a high mass of adsorbent which then lessens the surface area of STL exposed to target analytes for the adsorption to occur (Rovani et al., 2014). Thus, 5 mg of STL dosage was applied throughout this research.

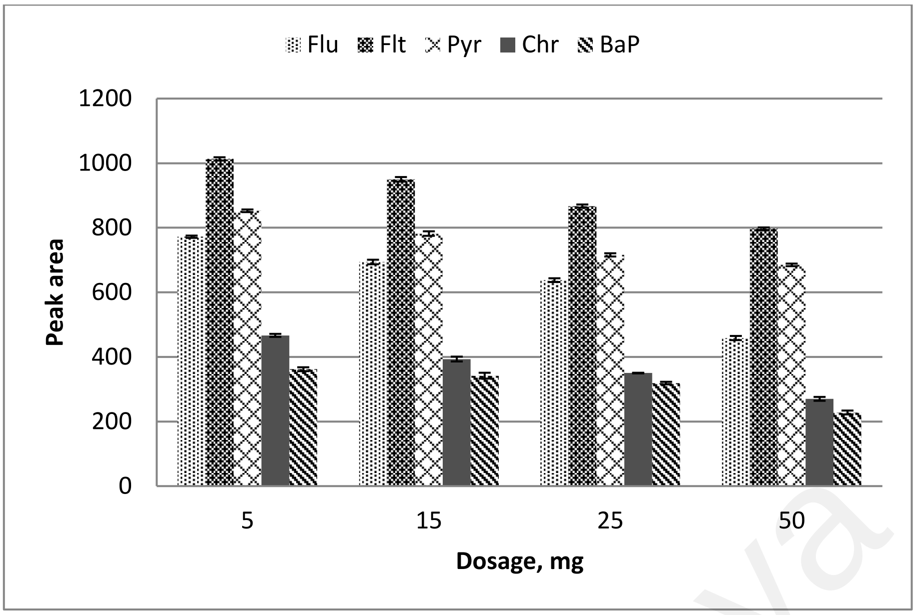


Figure 4.5: The effect of adsorbent dosage on the extraction efficiency of PAHs. (Extraction conditions: Eluent: hexane; Adsorption and Desorption time: 10 minutes; Volume of eluent: 500 µL; Volume of the sample: 5 mL)

4.2.3 Adsorption time

Adsorption time is the time given for analyte and adsorbent particles to interact. Adequate time should be given to attain adsorption equilibrium in order to achieve optimum extraction. This third parameter was investigated by varying the sonication time of adsorbent in the sample solution. The time was set in a series of 2, 5, 10, 12, and 15 minutes. This parameter is highly dependent on the partition coefficient of analytes in samples and adsorbent, which will further affect the number of analytes adsorbed from the aqueous sample (Naing et al., 2016b). **Figure 4.6** shows that the partitioning between two-phases happened rapidly until 12 minutes of adsorption time happened. After 12 minutes, the partitioning decreases, this can be explained as the adsorption time increases the saturation of analytes happens on the STL surfaces and leads to back-diffusion of analyte from adsorbent surface (Basheer et al., 2010; Naing et al., 2016a). Back-diffusion happened when the agitation happens too long, the analytes will unattached from the solid surface and dissolve back into the sample solution. This phenomenon reported in other earlier findings (Hii et al., 2009). Thus, 12 minutes was selected as the best adsorption time for this proposed method and used in further investigation.

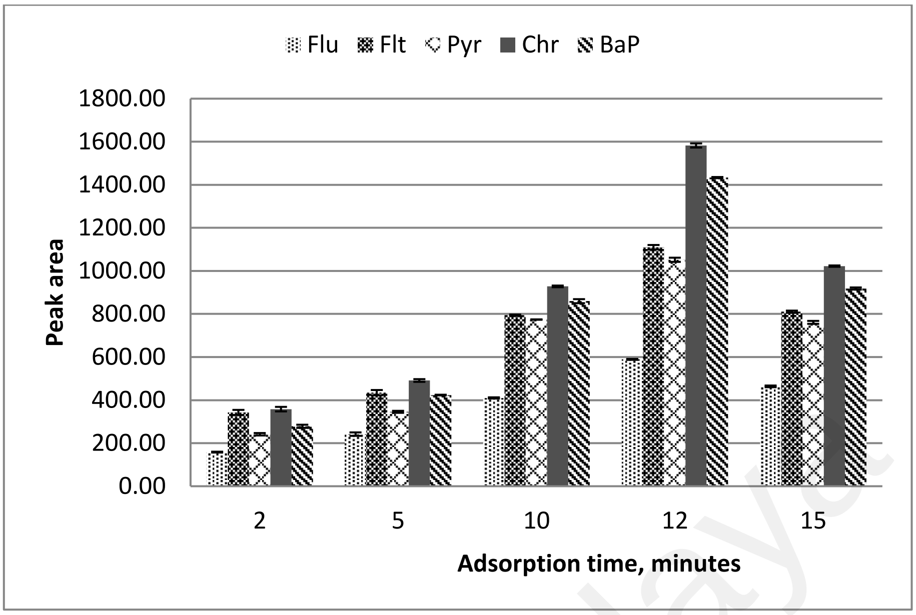


Figure 4.6: The effect of adsorption time on the extraction efficiency of PAHs. (Extraction conditions: Eluent: hexane; Adsorbent dosage: 5 mg; Desorption time: 10 minutes; The volume of eluent: 500 µL; The volume of sample: 5 mL)

4.2.4 Desorption time

Adequate time should be given for all analyte to detach it from the adsorbent into the eluent. The effect of desorption time on extraction performances investigated by making a series of sonication time of 2 to 12 minutes. **Figure 4.7** shows as the time of desorption increases, the chromatography signal increases until 10 minutes and further decreases after 10 minutes. The decreases might due to prolonged extraction time that makes re-absorption of the analyte onto adsorbent ‘s active sites happen (Ge & Lee, 2011). This phenomenon makes the lesser analyte detected by GC-FID and it is also reported in other micro-extraction studies before (Moradi et al., 2019). It is important to select an optimum desorption time so that the extraction efficiency did not interrupt. Hence, 10 minutes was chosen as an efficient desorption time and used in further studies.

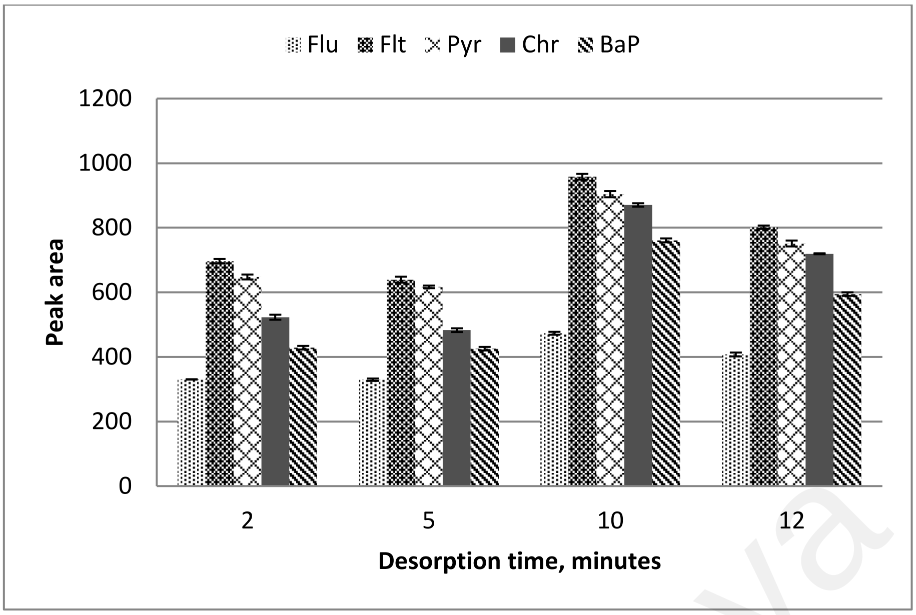


Figure 4.7: The effect of desorption time on the extraction efficiency of PAHs. (Extraction conditions: Eluent: hexane; Adsorbent dosage: 5 mg; Adsorption time: 12 minutes; The volume of eluent: 500 µL; The volume of sample: 5 mL)

4.2.5 Volume of eluent

The correlation of eluent volume with extraction efficiency was investigated. By fact, high enrichment of PAHs prefers a low volume of eluent (Sajid et al., 2016a). To investigate this parameter, the volume of eluent was set in a series of 500, 750, 1000, and 2000 µL. Based on the result in **Figure 4.8**, clearly shown that as the volume of eluent decreases the chromatography signal enhanced. This can be explained as lower eluent volume used, the PAHs will be concentrated in it but if a higher volume of eluent added the dilution will happen thus decrease the chromatography signal (Basheer et al., 2007). Thus, 500 µL is the minimum volume of eluent that can be used because the µ-SPE has to be completely dipped in the eluent solvent to make sure the detachment of PAHs from STL surfaces happens completely. Thus, the optimum eluent volume for the rest of the experiment fixed as 500 µL.

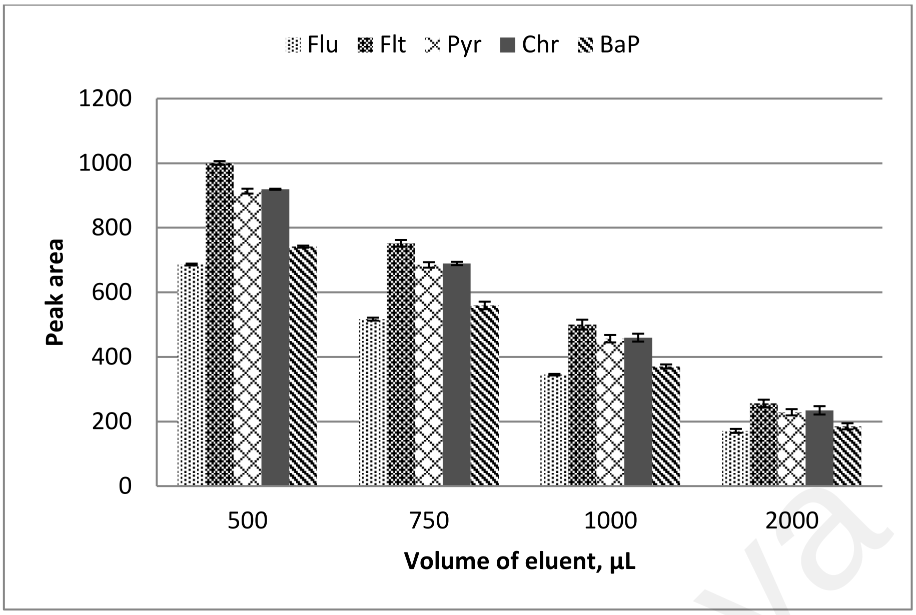


Figure 4.8: The effect of eluent volume on the extraction efficiency of PAHs. (Extraction conditions: Eluent: hexane; Adsorbent dosage: 5 mg; Adsorption time: 12 minutes; The desorption time: 10 minutes; The volume of sample: 5 mL)

4.2.6 Sample volume

The sample volume parameter is directly related to enrichment factor (EF) and the loading capacity of adsorbent (Basheer et al., 2009; Boon et al., 2019). An optimum EF and loading capacity can be accomplished with a usage of suitable sample volume. Too much of sample volume may distort adsorbent ‘s performances. The optimization step is compulsory to get the best extraction efficiency. Referring to **Figure 4.9** the analytical signal was increased till the sample volume is 5 mL and then gradually decreases. Sample volume lesser than 5 mL did not fulfill the loading capacity of STL, as there are vacant active sites of STL that did not occupy. While more than 5 mL, a large sample volume makes STL ‘s sites fully loaded with the analyte (Pichon, 2000) and the usage of huge sample volume is not practical. Thus, 5 mL of sample volume was selected as an optimized condition for the next experiment.

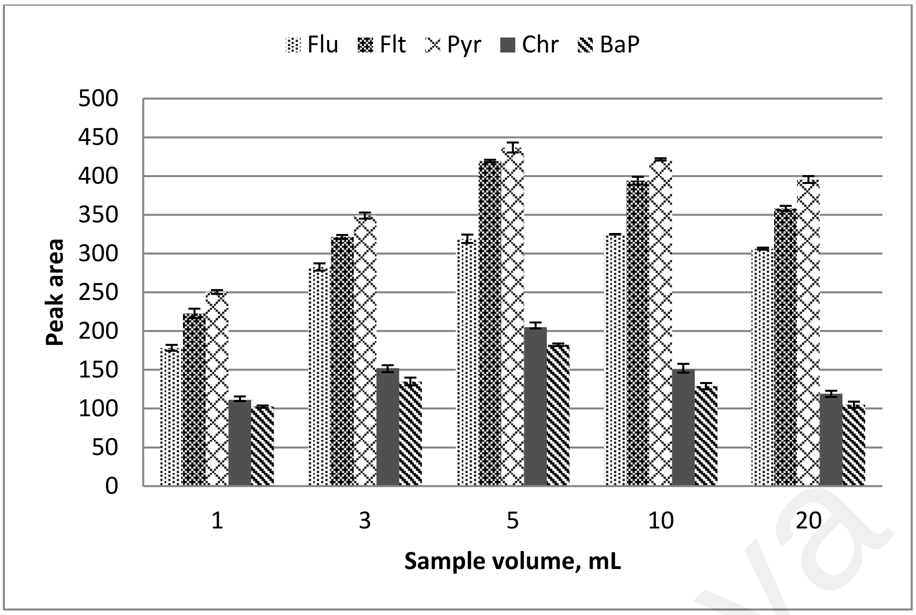


Figure 4.9: The effect of sample volume on the extraction efficiency of PAHs. (Extraction conditions: Eluent: hexane; Adsorbent dosage: 5 mg; Adsorption time: 12 minutes; Desorption time: 10 minutes; The volume of eluent: 500 µL)

Lastly from all the outcomes, the optimum condition of extraction procedure was selected and tabulated in **Table 4.1**.

Table 4.1: Optimum conditions for the extraction of PAHs by STL-µ-SPE

Extraction parameters	Optimum condition
Type of eluent	Hexane
Dosage of sorbent	5.0 mg
Adsorption time	12.0 minutes
Desorption time	10.0 minutes
Volume of eluent	500.0 µL
Volume of sample	5.0 mL

4.3 Carryover and reusability of STL-μ-SPE

Carryover can happen when the analytes did not detach completely from the STL surfaces. Thus, it is investigated by re-desorbed the dried μ-SPE device in 500 μL of hexane solvent and then injected into GC-FID. From the results, none of the analyte peaks observed on the chromatogram, stipulate there is no carryover and this device can be reused. Then, a reusability study was done to investigate how many cycles of extraction can STL-μ-SPE bear without deteriorating and results are consistent. Thus, in this study, the performance of STL-μ-SPE was found to be stable up to 6 cycles (**Figure 4.10**). Referring to **Figure 4.10** the analytical signal of each analyte is consistent for till the sixth cycles of usage and then slightly decreases for the seventh cycle usage. In conclusion, one STL-μ-SPE device can be used up to six cycles of extraction with a consistent result.

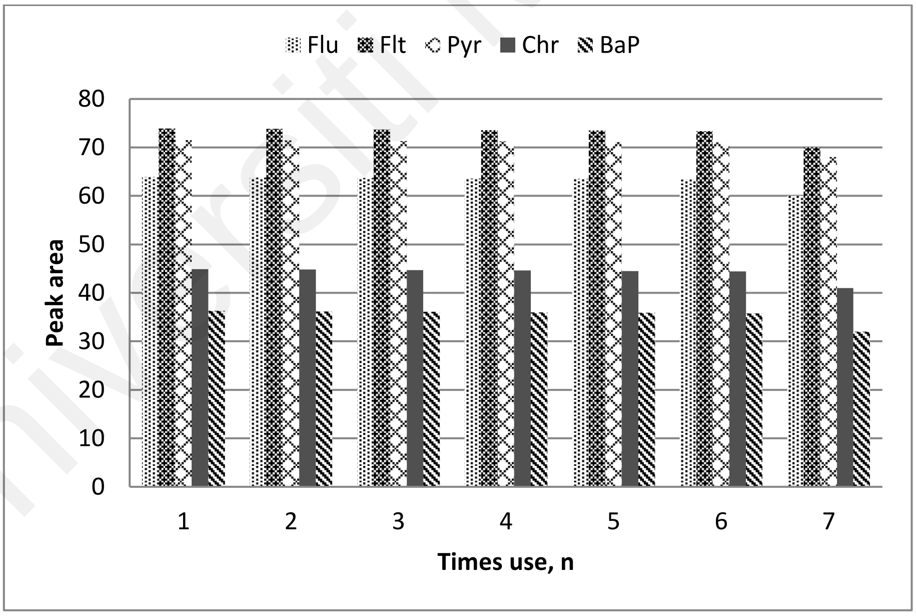


Figure 4.10: The reusability analysis of STL-μ-SPE.

4.4 Method validation

The proposed method, STL-μ-SPE coupled with GC-FID performances was validated by carrying a series experiment to determine its linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, pre-concentration factor (PF) and matrix effect. Matrix- match calibration curve for each PAH was prepared under the optimum condition and at least nine calibration levels of injection in four different matrices: water, rice, orange juice, and apple juice. The calibrations level of injection in water, orange juice, and apple juice are between 50.0 and 1000.0 ng mL^{-1} while for rice sample is 50 and 1000 μg kg^{-1} respectively with three replicates (n=3).

The linearity curve plotted for each PAH in each matrix exhibits a satisfactory coefficient of determination (R^2). Based on data from **Table 4.2**, **Table 4.3**, **Table 4.4**, and **Table 4.5**, in water the R^2 value is ranging from 0.9952 to 0.9983, in rice matrices is from 0.9957 to 0.9979, in orange juice is from 0.9953 to 0.9994, and in apple juice is from 0.9958 to 0.9981. The precision of this method investigated and found that the relative standard deviation (RSD%), the intra-day and inter-day at a spiking level of 700 ng mL^{-1} in water sample ranging from 5.23 to 7.76 and 6.90 to 10.86, respectively. This indicates the good reproducibility of this method in determining the concentration of PAHs in the water sample.

Also found that the LODs for water, rice, orange juice, and apple juice were recorded in a range of 8.47-27.34 ng mL^{-1}, 2.98-30.22 μg kg^{-1}, 9.54-29.15 ng mL^{-1}, and 9.30-55.95 ng mL^{-1}, respectively. Whereas the LOQs in each matrix respectively in the range of 28.23-91.14 ng mL^{-1}, 9.04-91.59 μg kg^{-1}, 31.81-97.16 ng mL^{-1}, and 31.00-186.54 ng mL^{-1}. The pre-concentration factor (PF) values for Flu, Flt, Pyr, Chr, and BaP found to be 22, 22, 18, 16 and 36 in water sample; 29, 43, 40, 19 and 25 in rice sample; 27, 33, 40, 20 and 23 in orange juice, and 29, 43, 40, 19 and 25 in apple juice. Based on the PF recorded, it can be concluded that STL as a waste adsorbent able to enhance the analytical signal of PAHs in samples.

While as for the matrix effect based on the comparison of the calibration curve in rice, orange juice, and apple juice versus the calibration curve in water by assuming there are no matrix effects in the water. The matrix effect in rice, orange juice, and apple juice was in the range of 1.34 % to 3.47 %, 0.57 % to 1.23 %, and 0.61 % to 1.66 %, respectively. The gradient difference of the calibration curves does not differ much indicating there is not much matrix effect in the different sample matrix. Besides that, by the preparing matrix-matched calibration curve, the matrix effects will be minimized.

Table 4.2: Analytical performances of STL-µ-SPE method in water matrices

Water	Linearity (ng mL^{-1})	R^2	RSD% (n=6)		LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Preconcentration Factor (PF)	Recovery (%) PAHs spiked (ng mL^{-1})(n=3)			
			Intraday	Interday				400	RSD (%)	700	RSD (%)
Flu	100-1000	0.9957	6.65	9.61	27.34	91.14	21.82	109.2	6.4	99.9	7.3
Flt	50-1000	0.9969	7.76	8.43	8.47	28.23	21.67	108.8	1.7	116.1	7.6
Pyr	50-1000	0.9983	5.23	6.90	14.89	49.62	18.28	111.7	8.7	103.9	3.1
Chr	300-1000	0.9956	6.68	8.83	24.69	82.31	15.68	88.0	9.8	105.0	9.0
BaP	100-1000	0.9952	7.07	10.86	25.53	85.11	36.36	98.4	6.2	98.7	8.6

Table 4.3: Analytical performances of STL-µ-SPE method in rice matrices

Rice	Linearity (µg kg^{-1})	R^2	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Preconcentration Factor (PF)	Matrix effect (%)	Recovery (%) PAHs spiked (µg kg^{-1})(n=3)			
							400	RSD (%)	700	RSD (%)
Flu	100-1000	0.9979	30.22	91.59	29.16	1.34	96.9	4.9	96.7	6.6
Flt	100-1000	0.9957	5.28	15.99	42.93	2.44	97.4	1.9	94.2	2.7
Pyr	100-1000	0.9978	5.81	17.62	39.50	3.47	94.4	1.4	99.6	2.0
Chr	100-1000	0.9971	2.98	9.04	19.44	3.36	96.1	2.1	93.9	4.8
BaP	100-1000	0.9975	15.60	47.27	25.27	3.16	93.5	5.4	98.6	2.0

Table 4.4: Analytical performances of STL-µ-SPE method in orange juice matrices

Orange juice	Linearity (ng mL^{-1})	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Preconcentration Factor (PF)	Matrix effect (%)	Recovery (%) PAHs spiked (ng mL^{-1})(n=3)			
							400	RSD (%)	700	RSD (%)
Flu	100-1000	0.9963	26.68	88.93	26.98	1.22	104.8	2.3	114.3	4.3
Flt	100-1000	0.9970	9.54	31.81	32.77	1.23	105.4	2.1	112.2	3.1
Pyr	200-1000	0.9953	17.81	59.36	40.29	1.03	105.7	3.3	116.7	2.1
Chr	100-1000	0.9956	16.07	53.56	19.55	0.57	105.0	5.7	107.5	6.0
BaP	100-1000	0.9994	29.15	97.16	22.82	0.64	111.4	1.6	110.4	6.8

Table 4.5: Analytical performances of STL-µ-SPE method in apple juice matrices

Apple juice	Linearity (ng mL^{-1})	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Preconcentration Factor (PF)	Matrix effect (%)	Recovery (%) PAHs spiked (ng mL^{-1})(n=3)			
							400	RSD (%)	700	RSD (%)
Flu	100-1000	0.9981	25.25	84.16	29.16	1.46	107.6	5.2	105.0	2.9
Flt	100-1000	0.9980	9.30	31.00	42.93	1.26	107.6	3.9	109.8	2.8
Pyr	100-1000	0.9964	11.04	36.80	39.50	1.66	102.9	1.4	109.1	2.4
Chr	100-1000	0.9947	10.49	34.95	19.44	0.87	101.6	2.5	105.0	1.0
BaP	200-1000	0.9958	55.96	186.54	25.27	0.61	103.1	6.5	107.3	1.2

The performances of the proposed approach were investigated in terms of relative recovery. Two known concentrations of PAHs analyte (400 and 700 ng mL^{-1}/ µg kg^{-1}) spiked into all sample matrices under optimum extraction condition, then the recovery of each PAH was recorded in term of a percentage to access the affectivity of STL-µ-SPE to extract PAHs from the sample matrices.

Referring to **Table 4.2**, **Table 4.3**, **Table 4.4**, and **Table 4.5**, the percentage of PAHs analyte recovered back from the spiked water, rice, orange juice, and apple juice were in the range of 88.0 % to 116.1 % with RSD of 1.7 to 9.8 %, 93.5 % to 99.6 % with RSD of 1.4 % to 6.6 %, 104.8 % to 114.3 % with RSD of 1.6 % to 6.8 % and 101.6 % to 109.8 % with RSD of 1.0 % to 6.5 %, respectively. The recovery percentages were positively acceptable, as the extraction efficiency is stable in all sample matrices and STL-µ-SPE performance does not affect by the difference of analyte concentration in the sample solution.

Figure 4.11 shows the GC-FID chromatogram of a blank solvent compared with the chromatogram of blank solvent with the addition of the teabag filter. This comparison is done to shows that, the teabag filter did not leech out any signal that can disrupt all five PAH target analyte's signals. While **Figure 4.12** shows the STL's leaching analysis result. STL-μ-SPE applied in non-spiked deionized water and based on the chromatogram result, there is no significant peaks observed. Thus concluded, STL did not leach out any PAHs that can obstruct our study. This fact is crucial because tea leaves may already be contaminated by PAHs through air deposition or from its production process (Londoño et al., 2015). Lin et al. (2006) stated that after tea leaves brewed for a few times the PAHs infused into the solution will decrease and this is proven by the GC-FID chromatogram of blank tea leaves. The steps of boiling the STL for few times in the preparations step do help in eliminating the PAHs that may exist in the STL, hence proven that the STL used were free from PAHs.

Other than that, **Figure 4.13** is provided to show the effectiveness of STL-μ-SPE to enhance the analytical signal of PAHs in the water sample. Chromatogram (A) shows the analytical peak of PAHs without the usage of STL as adsorbent and (B) after the implementation of the STL-μ- SPE technique, as seen all fives PAHs analytes peak enhanced. Concluded, STL has successfully pre-concentrated PAHs in water, rice, orange, and apple juice.

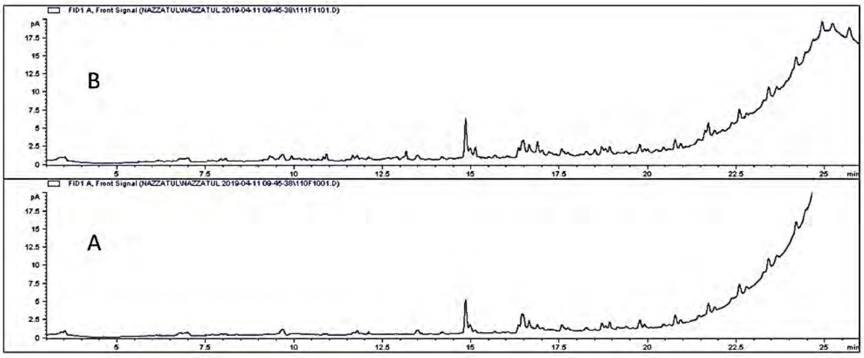


Figure 4.11: The GC-FID chromatogram of teabag filter leaching analysis. A) Blank solvent B) the addition of tea bag filter into the non-spiked solvent

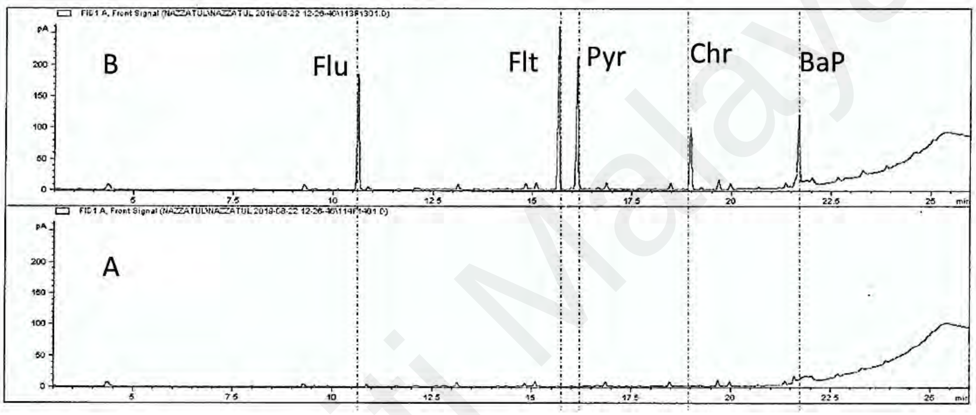


Figure 4.12: The GC-FID chromatogram of STL leaching analysis. STL-µ-SPE applied in A) non-spiked deionized water and B) deionized water spiked with 5 µg mL^{-1} of PAHs

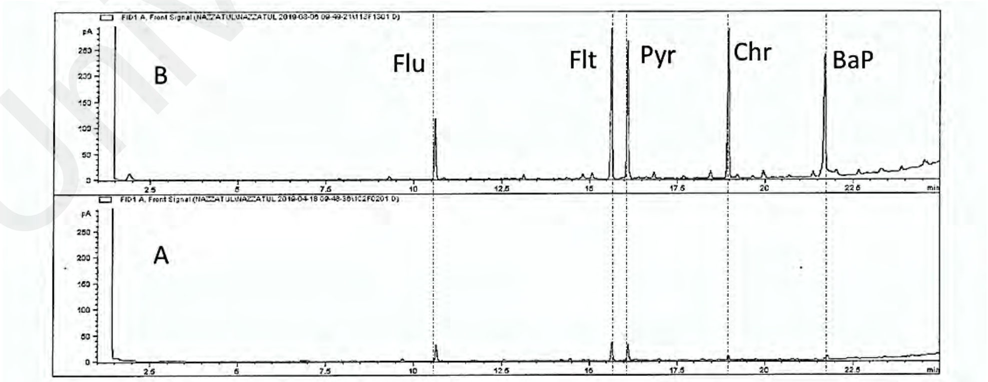


Figure 4.13: The GC-FID chromatogram after the STL-µ-SPE. A) 5 µg mL^{-1} of PAHs spiked into methanol and B) after the STL-µ-SPE step of 5 µg mL^{-1} PAHs spiked into a water sample.

4.5 Real sample analysis

Then the applicability of this proposed method was accessed by applying it in real sample matrices. A total of 36 samples was investigated including seven rivers, one well, one tap water sample, nine types of different rice samples, nine orange juice samples, and nine apple samples. Samples differentiated by the source of water collected, type of rice sample used and while for the juices by the location of the fruits bought. Variety of samples investigated to have a wide range of samples been screened for PAHs contamination. Triplicate extractions are done for each sample (n=3).

Table 4.6 shows the quantitative results on the determination of PAHs in water, rice, orange, and apple juice. Only Flt detected in eight out of nine water samples with concentrations more than the LOQ and LOD. Other four analytes not detected in any of the samples, they might be really absence in sample or its concentration lower than the quantification range of this method. Then the carcinogenic potency of the detected PAHs was expressed in terms of Toxic Equivalent Factor (TEQ). This parameter combines all PAHs detected in certain samples and its potency assessment calculated. TEQ calculated by using **Equation 4.1** (Nisbet & Lagoy, 1992):

$$TEQ = \sum PAHi \times TEFi \qquad \textbf{Equation 4.1}$$

where PAH_i is the concentration of PAH $congener_i$; TEF_i is the toxic equivalent factor of PAH $congener_i$ and TEQ is the toxic equivalents of the reference compound.

The highest and lowest values of TEQ recorded by samples 3 and 4 are 0.10893 and 0.04302, respectively. Out of five types of PAHs investigated, only Flt detected in the water samples. Sample 3 and sample 5 recorded 108.93 and 101.34 ng mL^{-1} of Flt detected, sample taken from two different rivers which surrounded by industrial factories and located at the roadside. The contamination might come from the burning of coal, cigarette smoke, and industrialization work (Pohanish, 2017) which possibly occurred around the sampling sites. The detection of Flu in the real samples shows that this proposed method suitable for real sample applications.

Table 4.6: PAHs determinations in water, rice, orange and apple juice by STL-µ-SPE coupled with GC-FID

	Founded (ng mL^{-1})					
Samples	**Flu**	**Flt**	**Pyr**	**Chr**	**BaP**	**TEQ**
Water						
S1	nd	45.24	nd	nd	nd	0.04524
S2	nd	69.72	nd	nd	nd	0.06972
S3	nd	108.93	nd	nd	nd	0.10893
S4	nd	43.02	nd	nd	nd	0.04302
S5	nd	101.34	nd	nd	nd	0.10134
S6	nd	< LOQ	nd	nd	nd	–
S7	nd	91.52	nd	nd	nd	0.09152
S8	nd	61.17	nd	nd	nd	0.06117
S9	nd	50.45	nd	nd	nd	0.05045
Orange juice						
S1	nd	nd	nd	nd	nd	–
S2	nd	nd	nd	nd	nd	–
S3	nd	nd	nd	nd	nd	–
S4	nd	nd	nd	nd	nd	–
S5	nd	nd	nd	nd	nd	–
S6	nd	nd	nd	nd	nd	–
S7	nd	nd	nd	nd	nd	–
S8	nd	nd	nd	nd	nd	–
S9	nd	nd	nd	nd	nd	–
Apple juice						
S1	nd	nd	nd	nd	nd	–
S2	nd	nd	nd	nd	nd	–
S3	nd	nd	nd	nd	nd	–
S4	nd	nd	nd	nd	nd	–
S5	nd	nd	nd	nd	nd	–
S6	nd	nd	nd	nd	nd	–
S7	nd	nd	nd	nd	nd	–
S8	nd	nd	nd	nd	nd	–
S9	nd	nd	nd	nd	nd	–
Rice	Founded (µg kg^{-1})					
S1	nd	nd	nd	nd	nd	–
S2	nd	nd	nd	nd	nd	–
S3	nd	nd	nd	nd	nd	–
S4	nd	nd	nd	nd	nd	–
S5	nd	nd	nd	nd	nd	–
S6	nd	nd	nd	nd	nd	–
S7	nd	nd	nd	nd	nd	–
S8	nd	nd	nd	nd	nd	–
S9	nd	nd	nd	nd	nd	–

nd = not detected

4.5 Adsorption phenomenon

Well-known that, STL ‘s cell wall mainly made-up of condensed tannin, cellulose, hemicellulose, and lignin (Thapak et al., 2015). Cellulose and hemicellulose provided a space for strong hydrogen bonding to happen through its polyhydroxy and polycarboxylic structure (Huang et al., 2006) while lignin provides a relatively hydrophobic region that able to attracts PAHs (Crisafully et al., 2008). This adsorption phenomenon has been proved based on FESEM images and FTIR spectra as in **Figure 4.14** and in **Figure 4.15**. Based on the FESEM image (A) in, it shows the morphology of STL before it is exposed to PAHs, (B) shows PAHs form a white layer that covering STL and lastly, after desorption, all PAHs detached and STL surface become clear. The white layer covering the STL assumed to be PAHs as it appeared after adsorption steps. Other than that, from the FTIR spectra in, the C=C bond skeletal stretching which representing aromatic ring becomes more intense as PAHs adsorb on the STL surface supporting FESEM images before. This may happen due to the PAHs which adsorbed on the STL surface. Besides that, this specific peak was also shifted from 1618 to 1634 cm^{-1}, indicating that π- π interactions happen and play a big role in the adsorption of PAHs on STL surfaces (Wang et al., 2014c) as shown in **Figure 4.15**. This discovery supported the idea that π-π interactions play a role in the adsorption of PAHs on STL surfaces.

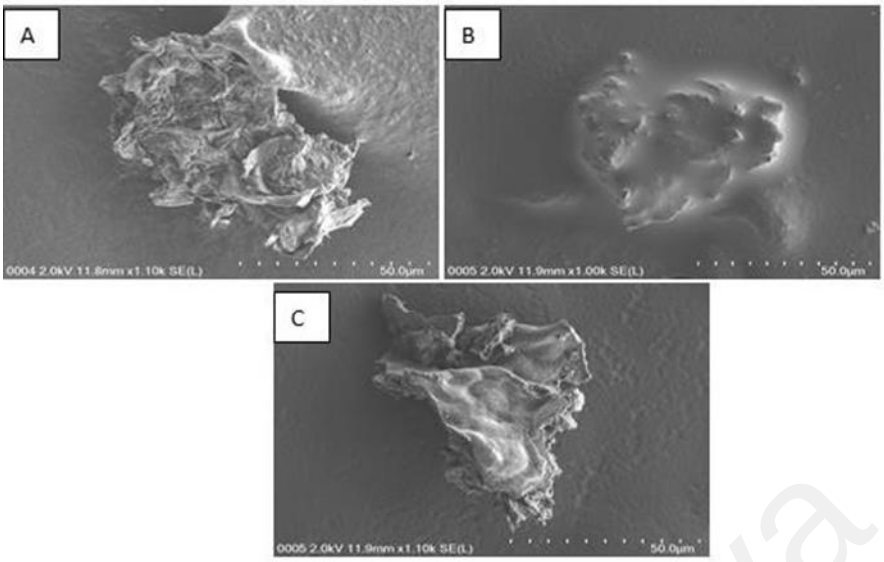


Figure 4.14: FESEM images of the adsorption mechanism of STL.
A) before adsorption, B) adsorption of PAHs and C) after desorption of PAHs.

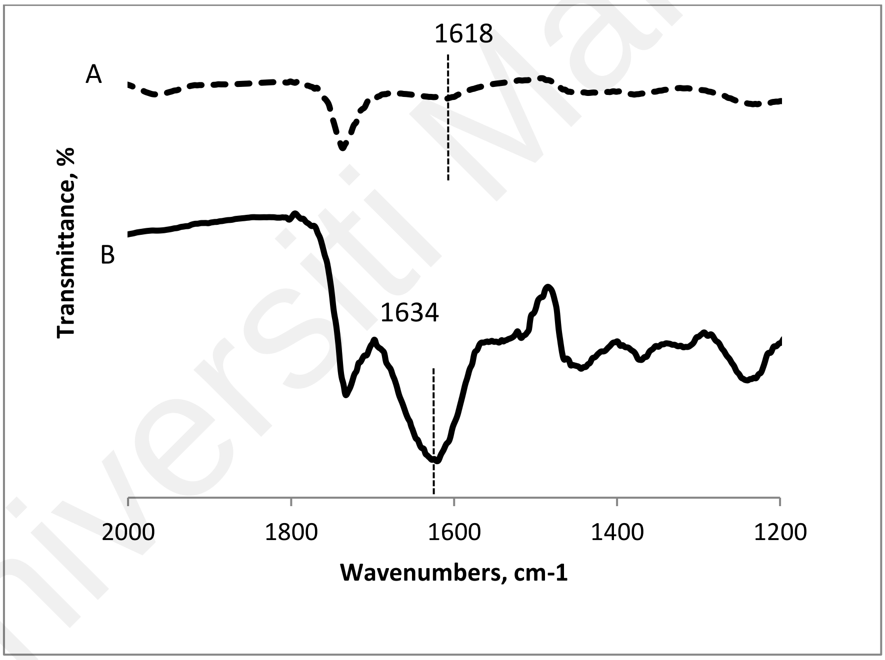


Figure 4.15: FTIR spectra of adsorption mechanism of STL. A) before the adsorption and B) after the adsorption of PAHs.

CHAPTER 5 : CONCLUSION AND FUTURE DIRECTION

5.1 Conclusion

Throughout this study spent tea leaves (STL) were successfully prepared and characterized by FTIR, FESEM, and EDX analysis. From the analysis done, STL was proved to have high surface area and mainly contain lignin, cellulose, hemicellulose, and polyphenols in it. These chemical compositions believed to be responsible for STL 's ability to adsorb and desorb PAHs in samples. Next, STL-μ-SPE coupled with the GC-FID technique was developed and then validated for the determination of PAHs in water, rice, orange juice, and apple juice sample. This method's precision determined and expressed in terms of the relative standard deviation (RSD%). Intraday and inter-day precision at a spiking level of 700 ng mL^{-1} in a water sample found to be in the range of 5.23 to 7.76 and 6.90 to 10.86, respectively. The LOD obtained for this method in water, rice, orange juice, and apple juice sample were recorded in a range of 8.47-27.34 ng mL^{-1}, 2.98-30.22 μg kg^{-1}, 9.54-29.15 ng mL^{-1}, and 9.30-55.95 ng mL^{-1}, respectively. While the LOQs of this method obtained in each matrix respectively in the range of 28.23-91.14 ng mL^{-1}, 9.04-91.59 μg kg^{-1}, 31.81-97.16 ng mL^{-1}, and 31.00-186.54 ng mL^{-1}. This proposed method also successfully implemented in real sample analysis (water, rice, orange, and apple juices) with good relative recovery and precision. The relative recovery of all PAHs spiked into all the sample matrices is in a range of 88.0 % to 116.1 %, with satisfactory precision (%RSD) between 1.0 % and 9.8 %. With all these, the objectives of the research were accomplished.

Though STL is an agricultural waste even without any modification it able to determine PAHs in the samples with an excellent relative recovery percentage. Besides that, with the combination with porous membrane protected μ-SPE this method able to have a good resistance on a dirty sample, lowered the matrix effect, and makes the application technique much easier and consumes lesser time. Thus, concluded that the

STL-μ- SPE coupled with the GC-FID technique is a green, bio-degradable, and cost-effective method which able to determine PAHs in samples.

5.2 Recommendation for future work

There were few recommendations in the next research, to enhance the extraction performances. First, we can attach an alkyl chain on STL's surfaces to make it more hydrophobic, thus able STL to have more hydrophobic interactions with PAHs, and in hopes, it can enhance STL 's ability to attract more hydrophobic PAHs. Second, the investigation effect of types of tea towards extraction performance of PAHs leaves should be investigated. This is because the chemical composition of other tea leaves may be slightly different and effecting the extraction performances differently. Lastly, there are a various type of natural adsorbent that can be utilized as PAHs adsorbent, for example, spent coffee, wood chips, papaya seeds, and many more. Higher lignin, cellulose, hemicellulose, polyphenols, and aliphatic carbon content in the natural sorbent will enhance the PAHs adsorption, thus increase the extraction performance.

REFERENCES

Abdel-Shafy, H. I., & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum, 25*(1), 107-123.

Agarry, S., Ogunleye, O., & Aworanti, O. (2013). Biosorption equilibrium, kinetic and thermodynamic modelling of naphthalene removal from aqueous solution onto modified spent tea leaves. *Environmental Technology, 34*(7), 825-839.

Agency for Toxic Substances and Disease Registry. (2020, August 7). Toxic Substances Portal - Polycyclic Aromatic Hydrocarbons ... Retrieved on 23 August 2020 from https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=122&tid=25

Aguinaga, N., Campillo, N., Vinas, P., & Hernández-Córdoba, M. (2007). Determination of 16 polycyclic aromatic hydrocarbons in milk and related products using solid-phase microextraction coupled to gas chromatography–mass spectrometry. *Analytica Chimica Acta, 596*(2), 285-290.

Al-Alam, J., Fajloun, Z., Chbani, A., & Millet, M. (2017). A multiresidue method for the analysis of 90 pesticides, 16 PAHs, and 22 PCBs in honey using QuEChERS–SPME. *Analytical and Bioanalytical Chemistry, 409*(21), 5157-5169.

Ali, A., Bilal, M., Khan, R., Farooq, R., & Siddique, M. (2018). Ultrasound-assisted adsorption of phenol from aqueous solution by using spent black tea leaves. *Environmental Science and Pollution Research, 25*(23), 22920-22930.

Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., & Kannan, K. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control, 22*(12), 2028-2035.

Amstaetter, K., Eek, E., & Cornelissen, G. (2012). Sorption of PAHs and PCBs to activated carbon: Coal versus biomass-based quality. *Chemosphere, 87*(5), 573-578.

Armstrong, B., Hutchinson, E., Unwin, J., & Fletcher, T. (2004). Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environmental Health Perspectives, 112*(9), 970-978.

Ashraf, M. W., & Salam, A. (2012). Polycyclic aromatic hydrocarbons (PAHs) in vegetables and fruits produced in Saudi Arabia. *Bulletin of Environmental Contamination and Toxicology, 88*(4), 543-547.

Awe, A. (2019). *Polycyclic Aromatic Hydrocarbons (PAHs) iIn the Diep And Plankenburg Rivers and potential remediation using charred Vitis vinifera (GRAPE) leaf litter* (Unpublished doctoral dissertation). Cape Peninsula University of Technology. Retrieved on 23 August 2020 from http://etd.cput.ac.za/handle/20.500.11838/2983

Awe, A. A., Opeolu, B. O., Olatunji, O. S., Fatoki, O. S., Jackson, V. A., & Snyman, R. G. (2020). Occurrence of PAHs in water samples of the Diep River, South Africa. *Water SA, 46*(1), 80-93.

Bajpai, S. K., & Jain, A. (2010). Sorptive removal of crystal violet from aqueous solution using spent tea leaves: Part I optimization of sorption conditions and kinetic studies. *Acta Chimica Slovenica, 57*(3), 751-757.

Basheer, C., Alnedhary, A. A., Rao, B. M., & Lee, H. K. (2009). Determination of carbamate pesticides using micro-solid-phase extraction combined with high-performance liquid chromatography. *Journal of Chromatography A, 1216*(2), 211-216.

Basheer, C., Alnedhary, A. A., Rao, B. M., Valliyaveettil, S., & Lee, H. K. (2006). Development and application of porous membrane-protected carbon nanotube micro-solid-phase extraction combined with gas chromatography/mass spectrometry. *Analytical Chemistry, 78*(8), 2853-2858.

Basheer, C., Chong, H. G., Hii, T. M., & Lee, H. K. (2007). Application of porous membrane-protected micro-solid-phase extraction combined with HPLC for the analysis of acidic drugs in wastewater. *Analytical chemistry, 79*(17), 6845-6850.

Basheer, C., Lee, J., Pedersen-Bjergaard, S., Rasmussen, K. E., & Lee, H. K. (2010). Simultaneous extraction of acidic and basic drugs at neutral sample pH: a novel electro-mediated microextraction approach. *Journal of Chromatography A, 1217*(43), 6661-6667.

Baudouin, C., Charveron, M., Tarroux, R., & Gall, Y. (2002). Environmental pollutants and skin cancer. *Cell Biology and Toxicology, 18*(5), 341-348.

Baxter, C. S., Hoffman, J. D., Knipp, M. J., Reponen, T., & Haynes, E. N. (2014). Exposure of firefighters to particulates and polycyclic aromatic hydrocarbons. *Journal of Occupational and Environmental Hygiene, 11*(7), D85-D91.

Boon, Y. H., Zain, N. N. M., Mohamad, S., Osman, H., & Raoov, M. (2019). Magnetic poly (β-cyclodextrin-ionic liquid) nanocomposites for micro-solid phase extraction of selected polycyclic aromatic hydrocarbons in rice samples prior to GC-FID analysis. *Food Chemistry, 278*, 322-332.

Boving, T. B., & Zhang, W. (2004). Removal of aqueous-phase polynuclear aromatic hydrocarbons using aspen wood fibers. *Chemosphere, 54*(7), 831-839.

Budhwani, N. (2015). Removal of polycyclic aromatic hydrocarbons present in tyre pyrolytic oil using low-cost natural adsorbents. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering,* 9, 186-190.

Cappiello, A., Famiglini, G., Palma, P., Pierini, E., Termopoli, V., & Trufelli, H. (2008). Overcoming matrix effects in liquid chromatography− mass spectrometry. *Analytical Chemistry, 80*(23), 9343-9348.

Caricchia, A. M., Chiavarini, S., & Pezza, M. (1999). Polycyclic aromatic hydrocarbons in the urban atmospheric particulate matter in the city of Naples (Italy). *Atmospheric Environment, 33*(23), 3731-3738.

Chefetz, B. (2003). Sorption of phenanthrene and atrazine by plant cuticular fractions. *Environmental Toxicology and Chemistry: An International Journal, 22*(10), 2492-2498.

Chen, B., Yuan, M., & Liu, H. (2011). Removal of polycyclic aromatic hydrocarbons from aqueous solution using plant residue materials as a biosorbent. *Journal of Hazardous Materials, 188*(1-3), 436-442.

Cheng, J., Zhang, X., Ma, Y., Zhao, J., & Tang, Z. (2019). Concentrations and distributions of polycyclic aromatic hydrocarbon in vegetables and animal-based foods before and after grilling: Implication for human exposure. *Science of The Total Environment, 690*, 965-972.

Choudhary, P., & Routh, J. (2010). Distribution of polycyclic aromatic hydrocarbons in Kumaun Himalayan Lakes, northwest India. *Organic Geochemistry, 41*(9), 891-894.

Çoruh, S., Geyikçi, F., & Nuri Ergun, O. (2011). Adsorption of basic dye from wastewater using raw and activated red mud. *Environmental Technology, 32*(11), 1183-1193.

Crisafully, R., Milhome, M. A. L., Cavalcante, R. M., Silveira, E. R., De Keukeleire, D., & Nascimento, R. F. (2008). Removal of some polycyclic aromatic hydrocarbons from petrochemical wastewater using low-cost adsorbents of natural origin. *Bioresource Technology, 99*(10), 4515-4519.

Dai, Y., Sun, Q., Wang, W., Lu, L., Liu, M., Li, J., . . . Xu, J. (2018). Utilizations of agricultural waste as adsorbent for the removal of contaminants: A review. *Chemosphere, 211*, 235-253.

De Jesus, J., Cunha, G. d. C., Cardoso, E., Mangrich, A., & Romão, L. (2017). Evaluation of waste biomasses and their biochars for removal of polycyclic aromatic hydrocarbons. *Journal of Environmental Management, 200*, 186-195.

Diggs, D. L., Harris, K. L., Rekhadevi, P. V., & Ramesh, A. (2012). Tumor microsomal metabolism of the food toxicant, benzo (a) pyrene, in Apc Min mouse model of colon cancer. *Tumor Biology, 33*(4), 1255-1260.

Dohmann, J. F., Thiäner, J. B., & Achten, C. (2019). Ultrasensitive detection of polycyclic aromatic hydrocarbons in coastal and harbor water using GC-APLI-MS. *Marine Pollution Bulletin, 149*, 110547.

Edwards, N. T. (1983). Polycyclic aromatic hydrocarbons (PAH's) in the terrestrial environment—a review. *Journal of Environmental Quality, 12*(4), 427-441.

Errais, E., Duplay, J., & Darragi, F. (2010). Textile dye removal by natural clay–case study of Fouchana Tunisian clay. *Environmental Technology, 31*(4), 373-380.

Eslamizad, S., Kobarfard, F., Javidnia, K., Sadeghi, R., Bayat, M., Shahanipour, S., . . . Yazdanpanah, H. (2016). Determination of benzo [a] pyrene in traditional, industrial and semi-industrial breads using a modified QuEChERS extraction, dispersive SPE and GC-MS and estimation of its dietary intake. *Iranian Journal of Pharmaceutical Research: IJPR, 15*, 165-174.

Galán-Cano, F., del Carmen Alcudia-León, M., Lucena, R., Cárdenas, S., & Valcárcel, M. (2013). Ionic liquid coated magnetic nanoparticles for the gas chromatography/mass spectrometric determination of polycyclic aromatic hydrocarbons in waters. *Journal of Chromatography A, 1300*, 134-140.

Ge, D., & Lee, H. K. (2011). Water stability of zeolite imidazolate framework 8 and application to porous membrane-protected micro-solid-phase extraction of polycyclic aromatic hydrocarbons from environmental water samples. *Journal of Chromatography A, 1218*(47), 8490-8495.

Gupta, H., & Gupta, B. (2016). Adsorption of polycyclic aromatic hydrocarbons on banana peel activated carbon. *Desalination and Water Treatment, 57*(20), 9498-9509.

Hafner, W. D., Carlson, D. L., & Hites, R. A. (2005). Influence of local human population on atmospheric polycyclic aromatic hydrocarbon concentrations. *Environmental Science & Technology, 39*(19), 7374-7379.

Hameed, B. H. (2009). Spent tea leaves: a new non-conventional and low-cost adsorbent for removal of basic dye from aqueous solutions. *Journal of Hazardous Materials, 161*(2-3), 753-759.

Heraldy, E., Osa, R., & Suryanti, V. (2016, February 9). A*dsorption of Procion Red MX 8B Using Spent Tea Leaves as Adsorbent*. Retrieved on 23 August 2020 from https://aip.scitation.org/doi/abs/10.1063/1.4941491

Hii, T. M., Basheer, C., & Lee, H. K. (2009). Commercial polymeric fiber as sorbent for solid-phase microextraction combined with high-performance liquid chromatography for the determination of polycyclic aromatic hydrocarbons in water. *Journal of Chromatography A, 1216*(44), 7520-7526.

Hu, H., Liu, S., Chen, C., Wang, J., Zou, Y., Lin, L., & Yao, S. (2014). Two novel zeolitic imidazolate frameworks (ZIFs) as sorbents for solid-phase extraction (SPE) of polycyclic aromatic hydrocarbons (PAHs) in environmental water samples. *Analyst, 139*(22), 5818-5826.

Huang, L., Boving, T. B., & Xing, B. (2006). Sorption of PAHs by aspen wood fibers as affected by chemical alterations. *Environmental Science & Technology, 40*(10), 3279-3284.

Hui, B. Y., Zain, N. N. M., Mohamad, S., Varanusupakul, P., Osman, H., & Raoov, M. (2020). Poly (cyclodextrin-ionic liquid) based ferrofluid: A new class of magnetic colloid for dispersive liquid phase microextraction of polycyclic aromatic hydrocarbons from food samples prior to GC-FID analysis. *Food Chemistry, 314*, #126214.

Hussain, S., Anjali, K., Hassan, S. T., & Dwivedi, P. B. (2018). Waste tea as a novel adsorbent: a review. *Applied Water Science, 8*(6), 165.

Hyder, M., Aguilar, L. L., Genberg, J., Sandahl, M., Wesén, C., & Jönsson, J. Å. (2011). Determination of polycyclic aromatic hydrocarbons (PAHs) from organic aerosols using hollow fiber micro–porous membrane liquid–liquid extraction (HF-MMLLE) followed by gas chromatography–mass spectrometry analysis. *Talanta, 85*(2), 919-926.

Jawad, A. H., Rashid, R. A., Mahmuod, R. M., Ishak, M. A. M., Kasim, N. N., & Ismail, K. (2016). Adsorption of methylene blue onto coconut (Cocos nucifera) leaf: optimization, isotherm and kinetic studies. *Desalination and Water Treatment, 57*(19), 8839-8853.

Juhasz, A. L., & Naidu, R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo [a] pyrene. *International Biodeterioration & Biodegradation, 45*(1-2), 57-88.

Kamaruzaman, S., Hauser, P. C., Sanagi, M. M., Ibrahim, W. A. W., Endud, S., & See, H. H. (2013). A simple microextraction and preconcentration approach based on a mixed matrix membrane. *Analytica Chimica Acta, 783*, 24-30.

Kameda, T. (2011). Atmospheric chemistry of polycyclic aromatic hydrocarbons and related compounds. *Journal of Health Science, 57*(6), 504-511.

Kang, S., & Xing, B. (2005). Phenanthrene sorption to sequentially extracted soil humic acids and humins. *Environmental Science & Technology, 39*(1), 134-140.

Kanimozhi, S., Basheer, C., Narasimhan, K., Liu, L., Koh, S., Xue, F., . . . Lee, H. K. (2011). Application of porous membrane protected micro-solid-phase-extraction combined with gas chromatography–mass spectrometry for the determination of estrogens in ovarian cyst fluid samples. *Analytica Chimica Acta, 687*(1), 56-60.

Karyab, H., Yunesian, M., Nasseri, S., Mahvi, A. H., Ahmadkhaniha, R., Rastkari, N., & Nabizadeh, R. (2013). Polycyclic aromatic hydrocarbons in drinking water of Tehran, Iran. *Journal of Environmental Health Science and Engineering, 11*(1), 25.

Khan, A., Wang, X., Gul, K., Khuda, F., Aly, Z., & Elseman, A. (2018a). Microwave-assisted spent black tea leaves as cost-effective and powerful green adsorbent for the efficient removal of Eriochrome black T from aqueous solutions. *Egyptian Journal of Basic and Applied Sciences, 5*(2), 171-182.

Khan, M. M. R., Rahman, M. W., Ong, H. R., Ismail, A. B., & Cheng, C. K. (2016). Tea dust as a potential low-cost adsorbent for the removal of crystal violet from aqueous solution. *Desalination and Water Treatment, 57*(31), 14728-14738.

Khan, R. J., Saqib, A. N. S., Farooq, R., Khan, R., & Siddique, M. (2018b). Removal of Congo red from aqueous solutions by spent black tea as adsorbent. *Journal of Water Chemistry and Technology, 40*(4), 206-212.

Khanna, M., Mathur, A., Dubey, A. K., McLaughlin, J., Moirangthem, I., Wadhwa, S., . . . Kumar, R. (2020). Rapid removal of lead (II) ions from water using iron oxide–tea waste nanocomposite–a kinetic study. *IET Nanobiotechnology, 14*(4), 275-280.

Kim, K.-H., Jahan, S. A., Kabir, E., & Brown, R. J. (2013). A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environment International, 60*, 71-80.

Kong, H., He, J., Gao, Y., Han, J., & Zhu, X. (2011). Removal of polycyclic aromatic hydrocarbons from aqueous solution on soybean stalk–based carbon. *Journal of Environmental Quality, 40*(6), 1737-1744.

Kuo, C., Hsu, Y., & Lee, H. (2003). Study of human exposure to particulate PAHs using personal air samplers. *Archives of Environmental Contamination and Toxicology, 44*(4), 0454-0459.

Lashgari, M., & Lee, H. K. (2016). Micro-solid phase extraction of perfluorinated carboxylic acids from human plasma. *Journal of Chromatography A, 1432*, 7-16.

Lavecchia, R., Pugliese, A., & Zuorro, A. (2010). Removal of lead from aqueous solutions by spent tea leaves. *Chemical Engineering Transactions, 19*, 73-78.

Lazim, Z. M., Mazuin, E., Hadibarata, T., & Yusop, Z. (2015). The removal of methylene blue and Remazol Brilliant Blue R dyes by using orange peel and spent tea leaves. *Jurnal Teknologi, 74*(11), 129-135.

Lin, D., Pan, B., Zhu, L., & Xing, B. (2007). Characterization and phenanthrene sorption of tea leaf powders. *Journal of Agricultural and Food Chemistry, 55*(14), 5718-5724.

Lin, D., Zhu, L., & Luo, L. (2006). Factors affecting transfer of polycyclic aromatic hydrocarbons from made tea to tea infusion. *Journal of Agricultural and Food Chemistry, 54*(12), 4350-4354.

Liu, L., Fan, S., & Li, Y. (2018). Removal behavior of methylene blue from aqueous solution by tea waste: kinetics, isotherms and mechanism. *International Journal of Environmental Research and Public Health, 15*(7), #1321.

Liu, X., & Korenaga, T. (2001). Dynamics analysis for the distribution of polycyclic aromatic hydrocarbons in rice. *Journal of Health Science, 47*(5), 446-451.

Londoño, V. A. G., Reynoso, C. M., & Resnik, S. L. (2015). Polycyclic aromatic hydrocarbons (PAHs) survey on tea (Camellia sinensis) commercialized in Argentina. *Food Control, 50*, 31-37.

Ma, T.-T., Shen, X.-F., Yang, C., Qian, H.-L., Pang, Y.-H., & Yan, X.-P. (2019). Covalent immobilization of covalent organic framework on stainless steel wire for solid-phase microextraction GC-MS/MS determination of sixteen polycyclic aromatic hydrocarbons in grilled meat samples. *Talanta, 201*, 413-418.

Malkoc, E., & Nuhoglu, Y. (2006). Fixed bed studies for the sorption of chromium (VI) onto tea factory waste. *Chemical Engineering Science, 61*(13), 4363-4372.

Manoli, E., & Samara, C. (1999). Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis. *TrAC Trends in Analytical Chemistry, 18*(6), 417-428.

Mazloomifar, A. (2013). Application of Dispersive Liquid-Liquid Microextraction with Graphite Furnace Atomic Absorption Spectrometry for Determination of Trace Amounts of Zinc in Water Samples. *International Journal of Analytical Chemistry, 2013*(2013), 743792.

Menzie, C. A., Potocki, B. B., & Santodonato, J. (1992). Exposure to carcinogenic PAHs in the environment. *Environmental Science & Technology, 26*(7), 1278-1284.

Miller, R. L., Garfinkel, R., Horton, M., Camann, D., Perera, F. P., Whyatt, R. M., & Kinney, P. L. (2004). Polycyclic aromatic hydrocarbons, environmental tobacco smoke, and respiratory symptoms in an inner-city birth cohort. *Chest, 126*(4), 1071-1078.

Mitra, S., & Mukherjee, T. (2018). Novel Low Cost Adsorbent for the Removal of Rhodamine B Dye from Wastewater. *International Journal of Engineering Research and Management, ISSN*, 2349-2058.

Mokgalaka, N., McCrindle, R., & Botha, B. (2004). Multielement analysis of tea leaves by inductively coupled plasma optical emission spectrometry using slurry nebulisation. *Journal of Analytical Atomic Spectrometry, 19*(10), 1375-1378.

Moradi, E., Ebrahimzadeh, H., & Mehrani, Z. (2019). Electrospun acrylonitrile butadiene styrene nanofiber film as an efficient nanosorbent for head space thin film microextraction of polycyclic aromatic hydrocarbons from water and urine samples. *Talanta, 205*(2019), #120080.

Mukhtar, N. H., & See, H. H. (2016). Carbonaceous nanomaterials immobilised mixed matrix membrane microextraction for the determination of polycyclic aromatic hydrocarbons in sewage pond water samples. *Analytica Chimica Acta, 931*, 57-63.

Müller, L., Gorecki, T., & Pawliszyn, J. (2006). Solid‐Phase Microextraction in Analysis of Pollutants in the Field. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*. Wiley, Chichester, UK (2000), pp. 3815-3831.

Naing, N. N., Li, S. F. Y., & Lee, H. K. (2016a). Evaluation of graphene-based sorbent in the determination of polar environmental contaminants in water by micro-solid phase extraction-high performance liquid chromatography. *Journal of Chromatography A, 1427*, 29-36.

Naing, N. N., Li, S. F. Y., & Lee, H. K. (2016b). Application of porous membrane-protected chitosan microspheres to determine benzene, toluene, ethylbenzene, xylenes and styrene in water. *Journal of Chromatography A, 1448*, 42-48.

Nasrollahpour, A., Moradi, S., & Baniamerian, M. (2017). Vortex-assisted dispersive solid-phase microextraction using ionic liquid-modified metal-organic frameworks of PAHs from environmental water, vegetable, and fruit juice samples. *Food Analytical Methods, 10*(8), 2815-2826.

Ngo, H. H., Guo, W., Zhang, J., Liang, S., Ton-That, C., & Zhang, X. (2015). Typical low cost biosorbents for adsorptive removal of specific organic pollutants from water. *Bioresource Technology, 182*, 353-363.

Nisbet, I. C., & Lagoy, P. K. (1992). Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regulatory Toxicology and Pharmacology, 16*(3), 290-300.

Nur-E-Alam, M., Mia, M. A. S., Ahmad, F., & Rahman, M. M. (2018). Adsorption of chromium (Cr) from tannery wastewater using low-cost spent tea leaves adsorbent. *Applied Water Science, 8*(5), 129.

Olivella, M. À., Jové, P., & Oliveras, A. (2011). The use of cork waste as a biosorbent for persistent organic pollutants–Study of adsorption/desorption of polycyclic aromatic hydrocarbons. *Journal of Environmental Science and Health, Part A, 46*(8), 824-832.

Owabor, C., Agarry, S., & Jato, D. (2012). Removal of naphthalene from aqueous system using unripe orange peel as adsorbent: effects of operating variables. *Desalination and Water Treatment, 48*(1-3), 315-319.

Pérez-Gregorio, M., García-Falcón, M., Martínez-Carballo, E., & Simal-Gándara, J. (2010). Removal of polycyclic aromatic hydrocarbons from organic solvents by ashes wastes. *Journal of Hazardous Materials, 178*(1-3), 273-281.

Pichon, V. (2000). Solid-phase extraction for multiresidue analysis of organic contaminants in water. *Journal of Chromatography A, 885*(1-2), 195-215.

Pohanish, R. P. (2017). *Sittig's handbook of toxic and hazardous chemicals and carcinogens* (Seventh ed., Vol. 1). United Kingdom, United States: William Andrew.

Razmi, H., Abdollahi, V., & Mohammad-Rezaei, R. (2016). Graphene quantum dots–eggshell nanocomposite to extract polycyclic aromatic hydrocarbons in water. *Environmental Chemistry Letters, 14*(4), 521-526.

Rovani, S., Censi, M. T., Pedrotti Jr, S. L., Lima, É. C., Cataluña, R., & Fernandes, A. N. (2014). Development of a new adsorbent from agro-industrial waste and its potential use in endocrine disruptor compound removal. *Journal of Hazardous Materials, 271*, 311-320.

Rozentale, I., Zacs, D., Perkons, I., & Bartkevics, V. (2017). A comparison of gas chromatography coupled to tandem quadrupole mass spectrometry and high-resolution sector mass spectrometry for sensitive determination of polycyclic aromatic hydrocarbons (PAHs) in cereal products. *Food Chemistry, 221*, 1291-1297.

Sajid, M. (2017). Porous membrane protected micro-solid-phase extraction: a review of features, advancements and applications. *Analytica Chimica Acta, 965*, 36-53.

Sajid, M., Basheer, C., & Mansha, M. (2016b). Membrane protected micro-solid-phase extraction of organochlorine pesticides in milk samples using zinc oxide incorporated carbon foam as sorbent. *Journal of Chromatography A, 1475*, 110-115.

Sajid, M., Basheer, C., Alsharaa, A., Narasimhan, K., Buhmeida, A., Al Qahtani, M., & Al-Ahwal, M. S. (2016a). Development of natural sorbent based micro-solid-phase extraction for determination of phthalate esters in milk samples. *Analytica Chimica Acta, 924*, 35-44.

Sanagi, M. M., Ling, S. L., Nasir, Z., Hermawan, D., Wan Ibrahim, W. A., & Naim, A. A. (2009). Comparison of signal-to-noise, blank determination, and linear regression methods for the estimation of detection and quantification limits for volatile organic compounds by gas chromatography. *Journal of AOAC International, 92*(6), 1833-1838.

Sánchez-González, J., García-Carballal, S., Cabarcos, P., Tabernero, M. J., Bermejo-Barrera, P., & Moreda-Piñeiro, A. (2016). Determination of cocaine and its metabolites in plasma by porous membrane-protected molecularly imprinted polymer micro-solid-phase extraction and liquid chromatography—tandem mass spectrometry. *Journal of Chromatography A, 1451*, 15-22.

Sánchez-González, J., Odoardi, S., Bermejo, A. M., Bermejo–Barrera, P., Romolo, F. S., Moreda–Piñeiro, A., & Strano-Rossi, S. (2019). HPLC-MS/MS combined with membrane-protected molecularly imprinted polymer micro-solid-phase extraction for synthetic cathinones monitoring in urine. *Drug Testing and Analysis, 11*(1), 33-44.

Sánchez-González, J., Salgueiro-Fernández, R., Cabarcos, P., Bermejo, A. M., Bermejo-Barrera, P., & Moreda-Piñeiro, A. (2017). Cannabinoids assessment in plasma and urine by high performance liquid chromatography–tandem mass spectrometry after molecularly imprinted polymer microsolid-phase extraction. *Analytical and Bioanalytical Chemistry, 409*(5), 1207-1220.

Sarria-Villa, R., Ocampo-Duque, W., Páez, M., & Schuhmacher, M. (2016). Presence of PAHs in water and sediments of the Colombian Cauca River during heavy rain episodes, and implications for risk assessment. *Science of the Total Environment, 540*, 455-465.

Schober, W., Lubitz, S., Belloni, B., Gebauer, G., Lintelmann, J., Matuschek, G., . . . Behrendt, H. (2007). Environmental polycyclic aromatic hydrocarbons (PAHs) enhance allergic inflammation by acting on human basophils. *Inhalation Toxicology, 19*(sup1), 151-156.

Silva, J., Morante, L., Demeke, T., Baah-Twum, J., & Navarro, A. E. (2019). Preparation and characterization of chemically-modified biomaterials and their application as adsorbents of Penicillin G. *Clean Technologies, 1*(1), 114-124.

Simoneit, B. R. (2002). Biomass burning—a review of organic tracers for smoke from incomplete combustion. *Applied Geochemistry, 17*(3), 129-162.

Singh, J., Boddula, R., & Jirimali, H. D. (2020). Utilization of secondary agricultural products for the preparation of value-added silica materials and their important applications: a review. *Journal of Sol-Gel Science and Technology,* 96, 15-33.

Sivakumaran, K., & Amarakoon, S. (2017). Bioactivity of fruit teas and tisanes–A review. *Journal of Pharmacognosy and Phytochemistry, 7*(1), 323-327.

Spietelun, A., Pilarczyk, M., Kloskowski, A., & Namieśnik, J. (2010). Current trends in solid-phase microextraction (SPME) fibre coatings. *Chemical Society Reviews*, 39(11), 4524-4537.

Srogi, K. (2007). Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environmental Chemistry Letters, 5*(4), 169-195.

Su, Y.-H., & Zhu, Y.-G. (2008). Uptake of selected PAHs from contaminated soils by rice seedlings (Oryza sativa) and influence of rhizosphere on PAH distribution. *Environmental Pollution, 155*(2), 359-365.

Sun, Y., & Wu, S. (2020). Analysis of PAHs in oily systems using modified QuEChERS with EMR-Lipid clean-up followed by GC-QqQ-MS. *Food Control, 109*, #106950.

Sun, Y., Wu, S., & Gong, G. (2019). Trends of Research on Polycyclic Aromatic Hydrocarbons in Food: A 20-year perspective from 1997 to 2017. *Trends in Food Science & Technology, 83*, 86-98.

Tee, T. W., & Khan, A. R. M. (1988). Removal of lead, cadmium and zinc by waste tea leaves. *Environmental Technology, 9*(11), 1223-1232.

Tesfagiorgis, K., Navarro, A. E., Chen, B. M., Herrera, N., Hernandez, J., González-Álvarez, Á., & Savane, O. S. (2020). Simulations of breakthrough curves for fixed-bed column adsorption of cobalt (II) ions on spent tealeaves. *Water Science and Technology: a Journal of the International Association on Water Pollution Research, 81*(11), 2410-2421.

Thapak, H. K., Sharma, J., Boudh, B., Banger, N., & Dwivedia, P. (2015). Adsorption of copper ions in aqueous media using tea waste and sawdust as an adsorbent. *IJIRST International Journal for Innovative Research in Science & Technology, 2*(3), 52-57.

Unwin, J., Cocker, J., Scobbie, E., & Chambers, H. (2006). An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK. *Annals of Occupational Hygiene, 50*(4), 395-403.

US EPA, . (2014, December). Priority Pollutant List. Retrieved on 23 August 2020 from https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf

Utkina, I. (2018, May 28). Global tea consumption and production driven by robust demand in China and India. *Food and Agricultural Organization of the United Nations*. Retrieved on 23 August 2020 from http://www.fao.org/news/story/en/item/1136255/icode/

Vyskocil, A., Fiala, Z., Chénier, V., Krajak, L., Ettlerova, E., Bukac, J., . . . Emminger, S. (2000). Assessment of multipathway exposure of small children to PAH. *Environmental Toxicology and Pharmacology, 8*(2), 111-118.

Wang, J., Chen, Z., & Chen, B. (2014c). Adsorption of polycyclic aromatic hydrocarbons by graphene and graphene oxide nanosheets. *Environmental Science & Technology, 48*(9), 4817-4825.

Wang, J., Liu, S., Chen, C., Zou, Y., Hu, H., Cai, Q., & Yao, S. (2014b). Natural cotton fibers as adsorbent for solid-phase extraction of polycyclic aromatic hydrocarbons in water samples. *Analyst, 139*(14), 3593-3599.

Wang, W., Chen, B., & Huang, Y. (2014a). Eggshell membrane-based biotemplating of mixed hemimicelle/admicelle as a solid-phase extraction adsorbent for carcinogenic polycyclic aromatic hydrocarbons. *Journal of Agricultural and Food Chemistry, 62*(32), 8051-8059.

Wong, S., Lee, Y., Ngadi, N., Inuwa, I. M., & Mohamed, N. B. (2018). Synthesis of activated carbon from spent tea leaves for aspirin removal. *Chinese Journal of Chemical Engineering, 26*(5), 1003-1011.

Wong, S., Tumari, H. H., Ngadi, N., Mohamed, N. B., Hassan, O., Mat, R., & Amin, N. A. S. (2019). Adsorption of anionic dyes on spent tea leaves modified with polyethyleneimine (PEI-STL). *Journal of Cleaner Production, 206*, 394-406.

Yagmur, E., Ozmak, M., & Aktas, Z. (2008). A novel method for production of activated carbon from waste tea by chemical activation with microwave energy. *Fuel, 87*(15-16), 3278-3285.

Yashin, A. Y., Nemzer, B. V., Combet, E., & Yashin, Y. I. (2015). Determination of the chemical composition of tea by chromatographic methods: a review. *Journal of Food Research, 4*(3), 56-87.

Younis, S. A., El-Gendy, N. S., El-Azab, W. I., & Moustafa, Y. M. (2015). Kinetic, isotherm, and thermodynamic studies of polycyclic aromatic hydrocarbons biosorption from petroleum refinery wastewater using spent waste biomass. *Desalination and Water Treatment, 56*(11), 3013-3023.

Zacs, D., Rozentale, I., Reinholds, I., & Bartkevics, V. (2018). Multi-Walled Carbon Nanotubes as Effective Sorbents for Rapid Analysis of Polycyclic Aromatic Hydrocarbons in Edible Oils Using Dispersive Solid-Phase Extraction (d-SPE) and Gas Chromatography—Tandem Mass Spectrometry (GC-MS/MS). *Food Analytical Methods, 11*(9), 2508-2517.

Zanjani, M. R. K., Yamini, Y., Shariati, S., & Jönsson, J. Å. (2007). A new liquid-phase microextraction method based on solidification of floating organic drop. *Analytica chimica acta, 585*(2), 286-293.

Zelinkova, Z., & Wenzl, T. (2015). The occurrence of 16 EPA PAHs in food–a review. *Polycyclic Aromatic Compounds, 35*(2-4), 248-284.

Zhang, N., Huang, C., Tong, P., Feng, Z., Wu, X., & Zhang, L. (2018). Moisture stable Ni-Zn MOF/g-C3N4 nanoflowers: A highly efficient adsorbent for solid-phase microextraction of PAHs. *Journal of Chromatography A, 1556*, 37-46.

Zhang, Y., Zhang, L., Huang, Z., Li, Y., Li, J., Wu, N., . . . Niu, Z. (2019). Pollution of polycyclic aromatic hydrocarbons (PAHs) in drinking water of China: Composition, distribution and influencing factors. *Ecotoxicology and Environmental Safety, 177*, 108-116.

Zhao, X., Fu, L., Hu, J., Li, J., Wang, H., Huang, C., & Wang, X. (2009). Analysis of PAHs in water and fruit juice samples by DLLME combined with LC-fluorescence detection. *Chromatographia, 69*(11-12), 1385-1389.

Zuorro, A., Lavecchia, R., Medici, F., & Piga, L. (2013). Spent tea leaves as a potential low-cost adsorbent for the removal of azo dyes from wastewater. *Chemical Engineering, 32*, 19-24.

Żwir-Ferenc, A., & Biziuk, M. (2006). Solid Phase Extraction Technique--Trends, Opportunities and Applications. *Polish Journal of Environmental Studies, 15*(5), 677-690.

LIST OF PUBLICATIONS AND PAPER PRESENTED

1. **Nazzatul**, A., Muggundha, R., & Sharifah, M. (2020). Spent tea leaves as an adsorbent for micro-solid-phase extraction of polycyclic aromatic hydrocarbons (PAHs) from water and food samples prior to GC-FID analysis. *Microchemical Journal,* 159, #105581.