THE ASSESSMENT OF SCREENING STUDY ON ORGANIC COMPOUNDS IN NON-HEATED AND HEATED LOCAL ELECTRONIC CIGARETTE LIQUID

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The price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand. This thesis is dedicated to my father, Rusli Bin Nizam who taught me that the best kind of knowledge to have been that which is learned for its own sake. It is also dedicated to my mother, Pn Zaharah Binti Ahmad who taught me that even the largest task can be accomplished if it is done one step at a time. To all my friends, thank you for your understanding and encouragement in my moment of crisis. Your friendship makes my life a wonderful experience. I cannot list all the name here, but you are always in my heart.

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ABSTRACT

Recently, the majority of smokers use electronic cigarettes (e-cigarettes). An e-cigarette generates vapor by heating an e-cigarette liquid (e-liquid) containing propylene glycol (PG) and vegetable glycerin (VG). At this point, the inhalation of e-liquid by the consumer may include hazardous organic compounds that create health concerns. Therefore, this study analyzed the chemical analysis of functional groups including pH in 37 e-liquids (non-heated and heated). The 37 samples were collected and purchased from the local retailers. Prior to functional group analysis, pH levels of all e-liquid samples were measured using a digital pH meter. The functional group of organic compounds in the samples was evaluated using Fourier transform infrared (FTIR) spectroscopy. The custom-made of vaping chamber is used to produce heated e-liquid samples. Next, a comparison of the functional group species between non-heated and heated samples are performed using FTIR analysis. A relationship between functional group of non-heated and heated e-liquids is validated using Pearson's correlation analysis. The pH of all e-liquids samples was in the range of 5.2 to 7.1 scale. FTIR spectra for non-heated and heated e-liquid are dominated by hydroxyl (OH-), alkane (C-H), alkene (C=C), carbonyl (C=O) and amide (N-H and C-N) groups. Significant peaks are observed at FTIR position of 3302 cm^{-1}, 2929 cm^{-1}, 1654 cm^{-1}, 1714 cm^{-1}, and 1554 cm^{-1}, respectively. There is also an increment in absorbance peak observed for both non-heated and heated e-liquids samples. These observations are supported by Pearson's correlation on the functional group of C=C, N-H and O-H for both non-heated and heated e-liquids samples in which the statistically significant is given by $r = 0.917$, $r = 0.567$ and $r = 0.417$ with p-value less than 0.05. Overall, this study revealed that e-liquids are mainly contained with hydroxyl, alkane, alkene, carbonyl and amide groups. This functional group confirmation were used for medical practitioners to predict the cause of health risk among the vaper’s patients in the future.

ABSTRAK

Baru-baru ini, majoriti perokok menggunakan rokok elektronik (e-rokok). E-rokok menghasilkan wap dengan memanaskan cecair e-rokok (e-cecair) yang mengandungi propilena glikol (PG) dan gliserin sayuran (VG). Pada ketika ini, penyedutan e-cecair oleh pengguna berkemungkinan mengandungi sebatian organik berbahaya yang menimbulkan kebimbangan kesihatan. Oleh itu, kajian ini menganalisis analisis kimia kumpulan berfungsi termasuk pH dalam 37 e-cecair (tidak dipanaskan dan dipanaskan). 37 sampel telah dikumpul dan dibeli daripada peruncit tempatan. Sebelum analisis kumpulan berfungsi, tahap pH semua sampel e-cecair diukur menggunakan meter pH digital. Kumpulan berfungsi sebatian organik dalam sampel telah dinilai menggunakan spektroskopi inframerah transformasi Fourier (FTIR). Ruang vaping yang dibuat khas digunakan untuk menghasilkan sampel e-cecair yang dipanaskan. Seterusnya, perbandingan spesies kumpulan berfungsi antara sampel tidak dipanaskan dan dipanaskan dilakukan menggunakan analisis FTIR. Hubungan antara kumpulan berfungsi bagi e-cecair tidak dipanaskan dan dipanaskan disahkan menggunakan analisis korelasi Pearson. pH semua sampel e-cecair adalah dalam julat 5.2 hingga 7.1 skala. Spektra FTIR bagi e-cecair tidak dipanaskan dan dipanaskan didominasi oleh kumpulan hidroksil (O-H), alkana (C-H), alkena (C=C), karbonil (C=O) dan amida (N-H dan C-N). Puncak yang ketara diperhatikan pada kedudukan FTIR 3302 cm^{-1}, 2929 cm^{-1}, 1654 cm^{-1}, 1714 cm^{-1}, dan 1554 cm^{-1}. Terdapat juga peningkatan dalam puncak penyerapan yang diperhatikan untuk kedua-dua sampel e-cecair tidak dipanaskan dan dipanaskan. Pemerhatian ini disokong oleh korelasi Pearson pada kumpulan berfungsi C=C, N-H dan O-H bagi kedua-dua sampel e-cecair tidak dipanaskan dan dipanaskan di mana signifikan secara statistik diberikan oleh $r = 0.917$, $r = 0.567$ dan $r = 0.417$ dengan nilai-p kurang daripada 0.05. Secara keseluruhan, kajian ini mendedahkan bahawa e-cecair terutamanya terkandung dengan kumpulan hidroksil, alkana, alkena, karbonil dan amida. Pengesahan kumpulan berfungsi ini boleh digunakan untuk pengamal perubatan meramalkan punca risiko kesihatan di kalangan pesakit vaper pada masa hadapan.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	percent
<	-	Less-than sign
>	-	Greater than sign
≤	-	Less-than and equal to sign
≥	-	Greater than and equal to sign
°C	-	Celsius
°F	-	Fahrenheit
µg/e-cigarette	-	Micrograms per e-cigarette
Ca^{2+}	-	Calcium ion
cm^{-1}	-	Reciprocal centimetre
g/cm^3	-	Gram per cubic centimetre
g/mol	-	Gram per mole
H^+	-	hydrogen ion
m	-	Meter
mAh	-	Milliamp Hour
mg	-	Milligram
mg/ml	-	Milligram per millilitre
ml	-	Millilitre
mm	-	millimetre
mm^2	-	Square millimetre
n	-	Natural number
ohm	-	resistance
Pa.s	-	Pascal-second
r	-	Pearson's Correlation
R^2	-	coefficient of discrimination
W	-	Watt

ZnSe	-	Zinc selenide
ASEAN	-	Association of Southeast Asian Nations
ATR	-	Attenuated Total Reflectance
CDC	-	Centers of Disease Control and Prevention
COPD	-	Chronic obstructive pulmonary diseases
DNA	-	Deoxyribonucleic acid
ENDS	-	Electronic nicotine delivery system
EVALI	-	E-cigarette, or Vaping Product, Use Associated Lung Injury
FDA	-	The United States Food and Drug Administration
FEMA	-	Flavor Extract Manufacturers Associations
FTIR	-	Fourier transform infrared (FTIR) spectroscopy
GC- FID	-	Gas chromatography with flame ionization detector
GC-MS	-	Gas chromatography with mass spectroscopy
GRAS	-	Generally recognized as safe
HPLC	-	High-performance liquid chromatography
HTP	-	Heat-not-burn tobacco product
HTS	-	High-throughput screening
IR	-	Infrared spectra
IUPAC	-	International Union of Pure and Applied Chemsitry
LED	-	Light-emitting diode
MDTCC	-	Ministry of Domestic, Trade, Cooperative and Consumerism
MEVTA	-	Malaysian E-Vaporisers and Tobacco Alternative Association
MOH	-	Ministry of Health
MOSTI	-	Ministry of Science, Technology and Innovation
NHMS	-	National Health and Morbidity Survey
NMR	-	Nuclear Magnetic Resonance
OSHA	-	Occupational Safety and Health Administration
PAH	-	Polycyclic aromatic hydrocarbon
PCA	-	Principal component analysis

PG	-	Propylene glycol
pH	-	Potential of hydrogen
PLS-DA	-	Partial Least Square – discriminant analysis
PM	-	Particulate matter
PPMC	-	Pearson product-moment correlation
RECESS	-	Research Centre for Soft Soil
RNS	-	Reactive nitrogen species
ROS	-	Reactive oxygen species
SAEC	-	Small airway epithelial cell
SD	-	Standard deviation
SE	-	Standard error
THC	-	Tetrahydrocannabinol
TSNA	-	Tobacco-specific nitrosamines
US	-	United States
UK	-	United Kingdom
UTHM	-	Universiti Tun Hussein Onn Malaysia
VG	-	Vegetable glycerin
VOC	-	Volatile organic compounds

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Smoking has become a severe public issue in the world. The number of smokers has been increasing from time to time (World Health Organization, 2018). The Association of Southeast Asian Nations (ASEAN) countries produced nearly 586 billion tobacco cigarettes and nearly 1.327 billion consumed by adults daily (Lian & Dorotheo, 2018). The number of smokers in Malaysia is estimated to be around 5 million consumers out of Malaysia's total population, 32.4 million (Lim *et al.*, 2018). Within these years, Malaysian governments have implemented regulations restricting this smoking habit among their citizens (Minister of Health, 2020). Due to this scenario, the implementation of smoking restriction in public and indoor areas has led all smokers to change their smoking habits. Based on Figure 1.1, the total sale of tobacco cigarettes in Malaysia decreases from 2011 until 2016 (Lian & Dorotheo, 2018). Hence, the National Health and Morbidity Survey (NHMS) stated that the popularity of the use of electronic cigarette (e-cigarette) had increased tremendously from 0.7% in 2011 to 10.9% in 2015 (Institute for Public Health (IPH) *et al.*, 2020). Meanwhile, the number of smokers has a tendency to increase from 2016 to 2021, indicating that there are dual users in the population (Ab Rahman *et al.*, 2019). These recent statistics indicate that most smokers have begun to change their lifestyle from tobacco cigarettes to e-cigarettes.

Here, the biggest concern is mainly focused on odour, dusk and air quality issues, especially in the indoor area (Canha *et al.*, 2019). Therefore, e-cigarettes device was introduced as one of other alternative to reduce air quality issue in the public and indoor area as well as to encourage smokers to reduce their smoking habits (Li *et al.*, 2020). Not only that, tobacco cigarette contains over 7000 chemical compounds and other poisonous substances in the smoke, such as tar, carbon monoxide, hydrogen

cyanide, etc. (Ratajczak *et al.*, 2021). Prior researchers claimed that e-cigarettes are less harmful than tobacco cigarettes because the e-cigarette contains fewer chemicals which are more hazardous than tobacco cigarettes (Balfour *et al.*, 2021; Nocella *et al.*, 2018). In fact, the British Department of Health also agreed that e-cigarettes are at least 95% less hazardous than tobacco cigarettes (McNeill *et al.*, 2018). This is due to the absence of combustion and reduced levels of harmful constituents in e-cigarette aerosol compared to the toxic smoke produced by tobacco cigarettes (Marques *et al.*, 2021). While tobacco smoke contains numerous harmful chemicals, including tar and carbon monoxide, e-cigarettes primarily produce an aerosol that consists of nicotine and a few other substances, which are typically found at much lower levels and are less harmful in comparison (Zhang *et al.*, 2018).

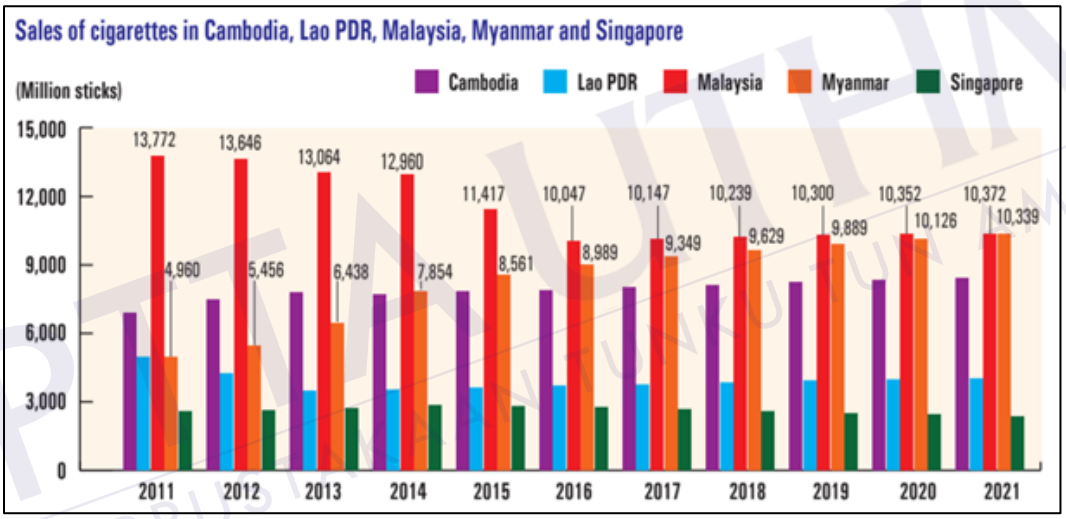

Figure 1.1: The bar chart of tobacco cigarettes sales in Cambodia, Lao PDR, Malaysia, Myanmar, and Singapore from 2011 to 2021 (Lian & Dorotheo, 2018).

The existence of e-cigarette started in the 18th century when Joseph Robinson submitted a patent for an electronic vaporizer that could be used with therapeutic chemicals (Robinson, 1930). Despite the fact that the patent was awarded, the gadget was never commercialized. Then, in 1963, an American named Herbert A. Gilbert filed a patent for a smokeless, non-tobacco cigarette to replace ordinary cigarette (Gilbert, 1965). The patent was awarded in 1965, however the device was never commercialised due to a lack of interest in smokeless cigarettes at the time. In the 20th century, the first e-cigarette was generally attributed to a Chinese pharmacist, Hon Lik, who worked at the Chinese company, Ruyan and patented the device in 2003

(Lik, 2013). The e-cigarette was introduced to the Chinese market in the following year (Löhler & Wollenberg, 2019).

To date, the use of e-cigarettes has extended across the globe, regardless of age and gender. The production of e-cigarettes has been developed, and various brands have been introduced to the market, for example, Juul (PAX Labs, San Francisco, CA, USA), Blu (Blu, Charlotte, NC, USA), NJOY (NJOY, Scottsdale, AZ, USA), etc. (K. E. Farsalinos *et al.*, 2018; Yeh *et al.*, 2016). The e-cigarette is known as a modern device powered by a battery that can heat up and convert the e-liquid containing nicotine into a vapor which will inhaled by the user (NIDA, 2020). The e-cigarette liquid (e-liquid) in the device's cartridge contains vegetable glycerin, propylene glycol, nicotine, and flavouring. The e-liquid is then heated in the atomizer (50°C - 250°C) and evaporated, producing an aerosol in the form of ultrafine particles with a flavour similar to smoke created by burning tobacco cigarettes (Zoccai *et al.*, 2020). As a result, combustion does not occur when using e-cigarettes, hence the term 'vapes' or 'vaping' is commonly used instead of smoking when referring to e-cigarettes (Marques *et al.*, 2021; Zoccai *et al.*, 2020).

E-cigarettes, including e-liquid, has been widely sold worldwide. Nevertheless, some countries like India, Brunei, Cambodia, Singapore, and Thailand have banned and restricted the sale of e-cigarettes as well as the use of e-cigarettes (Lian & Dorotheo, 2018). Furthermore, in 2015, Malaysia's National Fatwa Council deemed the use of e-cigarettes to be '*haram*' or strictly prohibited for Muslims. As a result, the governments of four Muslim-majority states, including Penang, Kedah, Johor, and Kelantan, have banned vaping (The Strait Times, 2015). In 2016, the government charged the Ministries of Health (MOH), Domestic Trade, Cooperatives, and Consumerism (MDTCC), and Science, Technology, and Innovation (MOSTI) with drafting a relating to the sale and use of e-cigarettes within two years (Bernama, 2016). After two years, the Malaysian federal government started regulating the ingredient of e-liquid and e-cigarette sales in 2018 (Whitehead, 2018). The following year, Dr Lee Boon Chye, the Deputy Health Minister, declared that e-cigarette manufacturing would be tightened in order to reduce the health risks associated with e-cigarette use among customers (Bernama, 2019).

To date, numerous studies have been performed in order to evaluate the safety or toxicity of e-cigarette using either qualitative or quantitative techniques, in vivo and in vitro cell structure for both e-liquid in liquid form or vapour form (Benam *et al.*,

2020; Farsalinos & Lagoumintzis, 2019; Li *et al.*, 2020; Palmisani *et al.*, 2020; Wavreil & Heggland, 2020). Farsalinos and Lagouminstzis (2019) conducted a study based on EU regulations and found that only one flavouring chemical in e-liquids reached toxic levels, while the concentrations of other chemicals were considerably lower. Palmisani et al. (2020) supported this finding by conducting experiments using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) to analyse BTEX (benzene, toluene, ethylbenzene, and xylene) contamination in e-liquids and gas chromatography-mass spectrometry-olfactometry (GC-MS-O) to identify specific flavouring additives responsible for particular flavours. However, there is no study that has been conducted on an investigation in determining the functional group of organic compounds found in e-liquid refill bottles (non-heated e-liquid) and e-liquids in e-cigarette cartridges (heated e-liquid) using infrared spectroscopy. Therefore, this study determines the functional group and organic compounds in non-heated and heated e-liquids using Fourier transform infrared (FTIR) spectroscopy.

1.2 Background of research

E-cigarette device has influenced e-cigarette users (known as "vapers") to use this device as a reason to replace tobacco cigarette (Wan Puteh *et al.*, 2018). Not only that, other reasons for the vapers to use e-cigarette are experimenting due to curiosity, variety of flavours, and following the trend (popularity) (Barakat *et al.*, 2021; Harrell *et al.*, 2017). The curiosity surrounding e-cigarettes can be attributed to individuals' desire to explore new technologies and experiences. Given that e-cigarettes are a relatively recent innovation which consist of a battery, a heating element (atomizer), a cartridge (container holding the e-liquid), a mouthpiece and an LED (Brown & Cheng, 2014). The operation of this device is to heat up (50°C – 250°C) the e-liquid in the cartridge in order to produce an aerosol. The main constituents of e-liquid are vegetable glycerin, propylene glycol, nicotine, and flavouring (Mohd Nawi *et al.*, 2020). Vapers can place the e-liquid in either a disposable cartridge or a refillable tank within the e-cigarette device. There are many e-liquids made in China, but some have been manufactured in the United States, Germany, and Europe (Bebenek *et al.*, 2022).

Recently, manufacturing of e-cigarettes in Malaysia has been developed, and various flavours of e-liquids have been made (Zulkifli *et al.*, 2020).

Nicotine, known as the primary addictive component in tobacco cigarettes, is also one of the components of e-liquids, which contain various levels of nicotine (Mahajan *et al.*, 2021). Previous studies found that the level of nicotine in e-liquid ranged from zero to 50 mg of nicotine (Gholap, Heyder, *et al.*, 2020; Shao & Friedman, 2020). Otherwise, the daily nicotine consumption is 37.6±17.7 mg, and the nicotine consumption of each cigarette is 1.04±0.36 mg (Benowltz & Jacob, 1984). Hence, according to previous research, the nicotine level of e-liquids ranged from 7–20 mg/ml, with an average of 12±4 mg/ml (El-Hellani *et al.*, 2018). This is demonstrated by nicotine forms that have two nitrogen groups in their chemical structure (pK_{a1} = 3.12 and pk_{a2} = 8.02), namely monoprotonated nicotine (nicotine salt) and free base nicotine (Duell *et al.*, 2020). The pH level influences nicotine absorption across cell membranes. Free base nicotine (pK_{a1}) increases absorption into the human body via epithelial tissue. Following that, e-liquids with a high freebase nicotine content will boost the user's body's nicotine intake rate (Shao & Friedman, 2020).

In addition, there has been a notable rise in the production of e-liquids worldwide, offering a wide array of unique flavours. Between 2013 and 2016, the number of flavours increased to a staggering 7,000 (Soule *et al.*, 2022). This increase highlights the availability of diverse flavour options, including tobacco, bubble gum, apple, grape, cotton candy, butterscotch, cheesecake, chocolate, vanilla, and coffee (Krüsemann *et al.*, 2018). Manufactures of e-liquid claimed that the formulation of e-liquid contents, including flavour compounds, are all food grade and 'generally recognized as safe' (GRAS). However, GRAS accreditation by the Flavor Extracts Manufacturers Association (FEMA) only applied to ingestion, not inhalation. FEMA now declared that the Expert Panel FEMA does not examine the use of flavouring compounds in tobacco products, including e-cigarettes and other non-human food products (Flavor and Extract Manufacturers Association, 2016). Hence, it is crucial to emphasize the investigation of the organic compounds constituting flavouring chemicals in e-liquids, considering that the flavourings commonly found in confectionery and food products are also likely to be used in e-cigarette products.

To date, numerous prior and on-going studies on chemical composition in e-liquid refill and e-liquid inside the cartridge have revealed that chemical compounds

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