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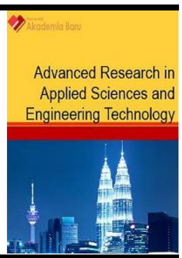

Identification of flavour chemicals and potentially harmful compounds in refill e-liquids sold in Malaysia

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ABSTRACT

E-cigarette has been marketed over years as one of alternative smoking cessation aid offered for consumer worldwide. The addition of flavouring in e-liquid has become a major factor that contributes to the appeal of using e-cigarette among a non-smoker and youngster has raised concerns due to possible risk and hazard outcomes in daily usage is remain unclear to date. In order to fully understand the e-cigarette attributes towards human health and environment; 17 e-liquid products locally sold were bought and analysed by gas chromatography-mass spectrometer (GC-MS) in order to determine the chemical constituent of e-liquid, especially the flavouring additive. A total of 19 out of 41 identified chemical compounds were detected as a new flavouring agent used in e-liquid products since there was no previous report on this. Many of the flavour chemicals possessed a risk of inhalation toxicity because they were classified as corrosive and/or irritant, with only a few having acute toxicity and the potential to cause cancer. Moreover, several chemical compounds to be concerned such as stimulant as well as opioid types were unexpectedly identified in the e-liquid. Thus, this finding indicate that the chemical constituent of e-liquid contained unknown risk of toxicity and further research is needed to fully understand the possibility of adverse effect in using e-cigarette.

Keywords:
E-cigarette; e-liquid; flavouring additive; youngster

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1. Introduction

Electronic cigarette or also be known as e-cig, electronic vaping device, personal vaporizer, and electronic nicotine delivery system (ENDS) is a battery operated device that emits visible aerosol through vaping activity where heating process occured with subsequent evaporation and condensation of solution consist of humectants acts as a solvent (i.e., propylene glycol and glycerine),

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with or without the addition of nicotine as well as flavourings. Initially, the purpose of e-cigarette has been invented as potentially safer alternative for smoking cessation aid by its mechanism that provide more rapid, flexible and effective nicotine delivery than a nicotine inhaler with similar sensation to inhaling tobacco smoke but without smoke since there is no combustion action involved thus eliminate the possibility of toxic byproduct of tobacco combustion to present [1]. However, vaped e-liquids can still undergo pyrolysis and pyrosynthesis due to e-cig heating component that can act as reactor operating within an oxygen containing atmosphere resulted in oxidative radical generation that lead to other toxicants formation such as reactive oxygen species (ROS), radicals, carbonyls and volatile organic compound (VOC)[2].

Contrary to its early goal of invention, the increasing trend of e-cig use worldwide especially among adolescent and non-never smoker without any indication in using e-cigarette as aid in smoking cessation or reduction has raised concerns since it may potentially increase the risk of nicotine dependence or eventually use of other tobacco products.In National E-cigarette Survey (NECS) of Malaysia [3], male and those with tertiary education in the age ranging among 18-24 years old were among the common e-cig user in conformity with studies conducted by Wong et al. [4] has reported that majority user was among young students of higher institutions (39.9%) and young professional and managers (36%) in the Selangor and Kuala Lumpur areas of Malaysia. Factors such as curiousity, peer influences, attractiveness of the product and availability of appealing flavours are among top reasons for the initiation of e-cig use and preference [5].

In 2014, around 7764 unique flavour names were reported available online with 242 new flavours added per month under 466 brands and continuously growing worldwide where Havermans et. al., (2021) had reported that nearly 20000 e-liquids with 250 unique flavour names were found in Dutch market solely in 2017 [6,7]. The flavoured e-liquid has been marketed commercially into different categories of flavours such as tobacco, mint, confectionary, fruits, beverage etc. The flavouring has been added as additives to enhance the taste of the e-liquid by combining several flavour chemicals to achieve desired flavour. While Food Extract Manufacturing Association (FEMA) has approved and evaluated the flavour ingredients as generally recognized as safe (GRAS), their approval only intended for ingestion means and not inhalation [8]. Due to the possibility of thermal degradation during e-liquid heating, the combination of different types of flavour chemicals may generate unknown harmful degradation product or toxicants as well as an unstable or new type of chemical compound could be formed harmful reaction product between the flavour chemicals and the base solvents in the aerosol e-cigarette. In study carried out by Bitzer et al. [9], the linalool, dipentene and citral are an example of flavour chemical combination that has been proven to produce high amount of free radical in aerosol during vaping when being combined. Moreover, with the vast number of e-liquids marketed locally, this may cause public health challenge due to the largely unknown toxicity profiles of e-cig as the flavour diversity increasing over times and current research has only been done on small proportion of available e-liquids leaving other flavours effect being unknown. In addition, as of now there is no any comprehensive study being conducted in Malaysia which focusing on chemical constituent of the commercially available e-liquid in current market and their related harmful possibility.

Thus, it is important and crucial to know the chemical component of flavoured e-liquid as it may give us ideas and understanding into the toxicity related to aerosol generation of e-cigarette based on transfer efficiency of the chemical compounds and their reactivity under condition implied in e-cig. In this study, we performed chemical profiling or screening of e-liquids to identify potentially harmful chemicals present especially flavour compounds in locally sold products.

2. Materials and Methods

2.1. Sampling of e-liquids for Chemical Compounds Detection

A total of 17 bottles of refill e-liquid were purchased online from local Malaysian distributor websites (Vapeempire.com & Vape69.com). Different type of brands and flavours such as fruity, creamy, sweet, beverage, mint and tobacco were randomly chosen with/or without nicotine. Nicotine concentration added were in a concentration range between 6 mg, 12 mg and 24 mg meanwhile PG/VG solvent content in the ratio ranged between 30:70, 50:50 and 70:30. All products were labeled accordingly and stored at room temperature and in dark condition until further analysis for flavour compounds, nicotine and other potentially harmful chemical compounds present in e-liquid.

Table 1
E-liquids listed according to flavour category

Flavour category	E-liquids	Nicotine (mg)	Brands
Tobacco	Tobacco Apple (TA),	6	MVH
	RY4,	24	Mr E's
	After 11 (DL)	12	Dinner Lady
Mint	Menthol (M)	24	Mr E's
	Lemon Ice (LI)	12	Mel Basics
	Root Beer Ice (RBI)	6	-
Dessert	Jam Berry Pancake (JBP)	12	Magnum
	Royal Blend (RB)	12	Finnesse
	Banana Muffin (BM)	6	Premium
			Steep Kreme
Fruit	Black Grape (BG)	6	Iceberg Blow
	Milky Strawberry (MS)	0	V2
	Den Osten Imperial (DOI)	24	The Barrels
Confectionary	Chewing Gum Strawberry (CGS)	6	Harum Manis
	Popcorn Caramello (PC)	12	
	Honeydew Bubblegum (HB)	6	Creamysuckerz
			-
Beverage	Mango Lassi (MLI)	12	Lassi Juice
	Mocha Latte (ML)	6	Aura

2.2 Profiling of Chemical Compounds in Refill e-liquid

Qualitative analysis of e-liquid by gas chromatograph-mass detector (GC-MS) was conducted on Agilent 7890A/5975C GC-MSD system using an Agilent J&W HP-5MS column (30 m x 250 µm x 0.25 µm) (Santa Clara, CA) and Helium as carrier gas. About 10µl of each refill e-liquids was dissolved in 90 µl of methanol before 1.0 µl aliquot of the diluted sample being injected into the GC with a split ratio of 5:1. The GC temperature program for analysis was: 60 °C for 2 min then 10 °C/min to 250 °C for 15 min, and the transfer line and ion source were held at 280 °, meanwhile 150 °C for quadrapole temperature. Full scan range of mass spectra was acquired from 30-300 amu. The chemical compound identification was performed using the NIST 2014 mass spectral database and AMDIS chromatography software. As for flavour compound identification, the results were further compared with data from FEMA Flavour Ingredient Library.

3. Results and Discussion

3.1. Identification of Flavour Compounds in e-liquid

Total of 41 flavour compounds were identified from 17 bottles of refill e-liquid purchased locally and listed as in Figure 1. The name abbreviation of flavoured e-liquids is positioned on the x-axis. In this study, flavour compounds such as methyl cyclopentenolone, ethyl maltol, ethyl vanillin, trimethylpyrazine, and butyric acid as well as vanillin and benzyl alcohol were conformed as commonly detected in e-liquid as in previous study and most frequently being added as flavouring ingredients in e-liquids from EU-CEG set data [6,9,11]. In addition, surprisingly 19 out of 41 flavour compounds detected has never been identified and reported as flavouring ingredients added in e-liquid before (Table A). This recent finding might be due to the myriad flavour profile of the tested e-liquids, considering the fact of e-liquid products availability is different across markets, particularly in Malaysia.

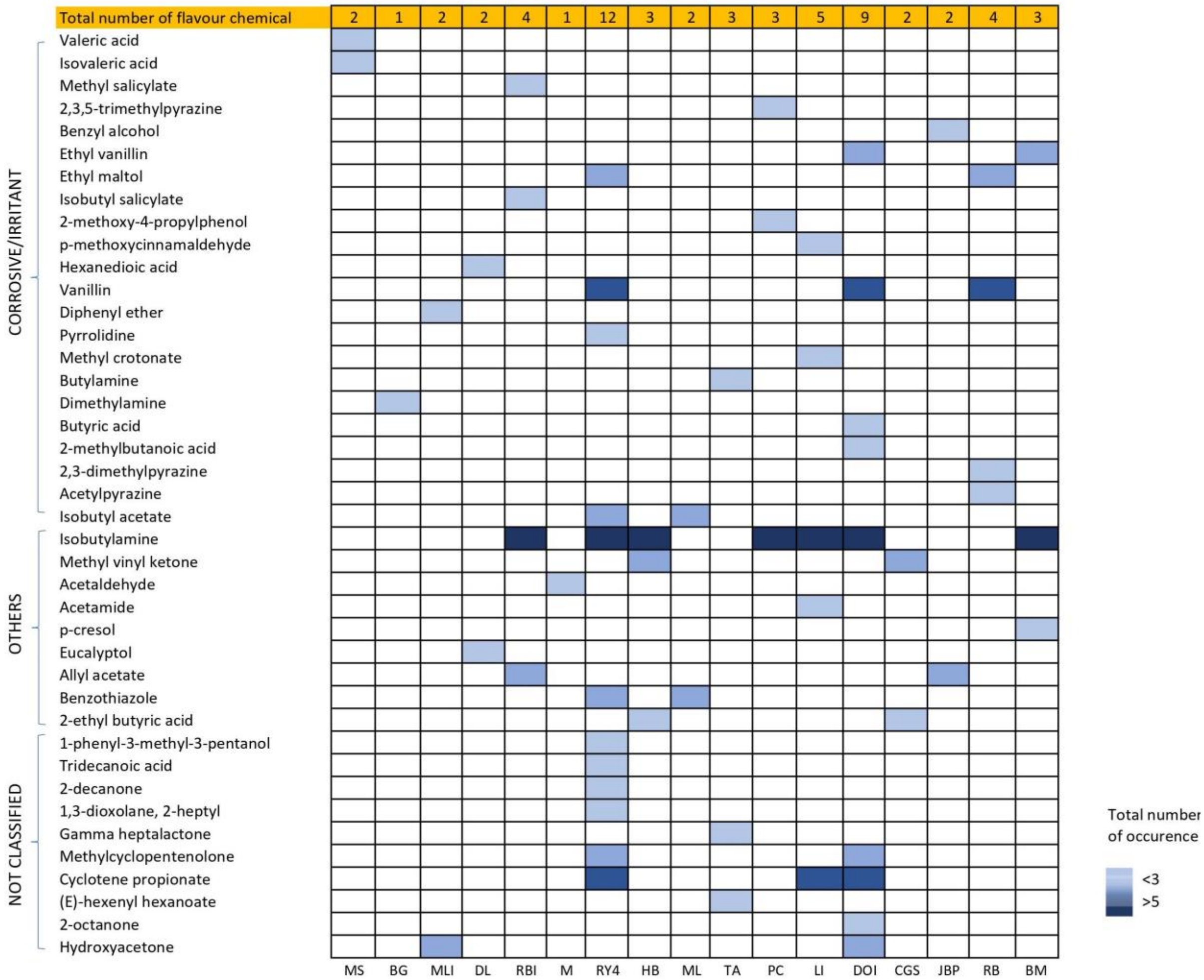

Fig. 1. Heat map of flavour compounds in 17 e-liquids. Chemicals are categorized according to toxicity on y-axis based on GHS Classification by OSHA and ECHA. The total number of flavor chemicals for each e-liquid is indicated at the top of column. The colour gradient on the right shows the total number of occurrences of each flavour chemicals

Meanwhile on the y-axis, the flavour compounds are individually classified into different group of health hazards according to GHS Classification in accordance with 29 CFR 1910 by Occupational Safety and Health Administration (OSHA) [10] and European Chemicals Agency (ECHA), whereby the categories applicable to the flavour compounds in our study included “Corrosive and/or Irritant”, “Others” and “Not Classified”. 22 out of 41 of flavour compounds were corrosive and/or irritant. As for “Others” category, the flavour compounds consisted of either one and/or more in combination of corrosive, irritant, acute toxic, carcinogenicity and aspiration hazard as their health hazards meanwhile there is no information available for “Not Classified” category.

The total number of flavour compouds of e-liquid is placed above each column respectively. RY4 had the highest total number of flavour chemicals (N=12) and followed by Den Osten Imperial (DOI) (N=9) while Black Grape (BG) and Menthol (M) flavoured e-liquid had the fewest flavour compounds (N=1). Next, the total number occurrence of flavour compounds is being distinct by different colour shades labeled as frequency varied. Isobutylamine, vanillin and cyclotene propionate were among having the most frequency of occurrence in the refill e-liquid products while some of the flavour compounds only appeared in one product. As the data shown, the total number of flavor compounds added into e-liquids was varied between products. It is suggested that the total complex or multi flavoured (i.e., RY4 and DOI) e-liquid might required more total combination of different flavour compounds in order to achieve the desired flavouring compared to single flavour e-liquid in this study. Herewith, the previous finding showed that a wide variety of flavouring chemicals was involved in e-liquid is validated [12,13].

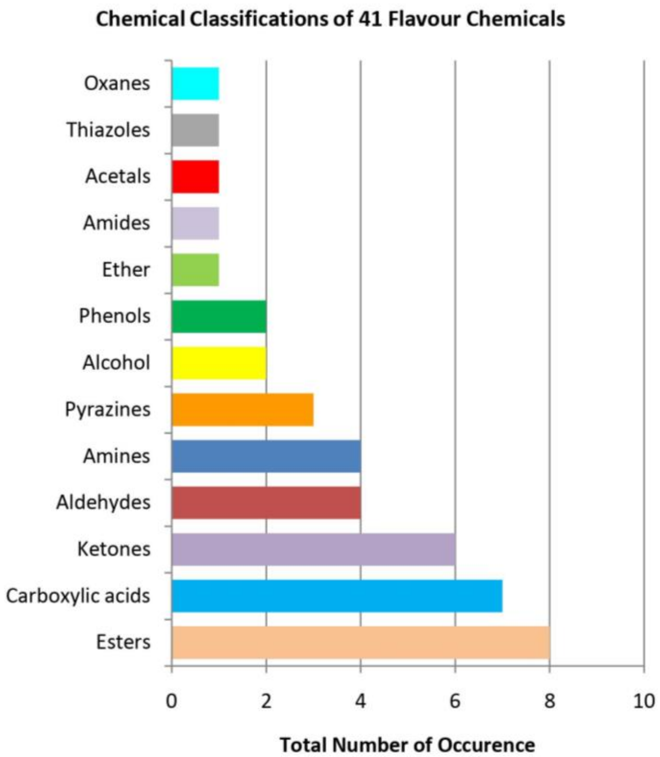

Fig. 2. The total occurrence numbers of flavor compounds according to their chemical classes

In addition, the data were analyzed in accordance with their respective chemical class (Figure 2). The most detected flavour compounds were esters followed by carboxylic acids, ketones, aldehydes and amine. However, addition of flavour compounds with potentially adverse health effects should be given attention; given the risk of inhalation toxicity is largely unknown and may cause inflammatory response in a long-term use even though they were certified as GRAS. Acetaldehyde as one of aldehyde compounds found in the e-liquid was found can directly impact the formation of free radical which eventually leads to oxidative stress to occur at cellular level [9]. Meanwhile, the flavour compounds such as vanillin, ethyl vanillin, benzyl alcohol, and 2-acetyl pyrazine as well as methyl salicylate which had high prevalence of occurrence in e-liquids also possessed acute toxicity as irritants on respiratory and skin. Besides that, the use of acetamide and eucalyptol as the flavouring agents has raised concerns since both compounds is known for their possibility in causing cancer and aspiration that can cause severe acute effects chemical pneumonia, pulmonary injury or death [14].

3.2 Identification of Nicotine in e-Liquid

Nicotine content of each e-liquid was diversed ranging from 0-24 mg/ml as labeled on the refill bottles by manufactures. In this study, the nicotine concentration as labeled was further confirmed by GCMS analysis. It was found that there was no nicotine detected in the refill e-liquid labeled as zero nicotine. However, there was no nicotine detected in 6 e-liquids which labeled as having 6 mg/ml and 24 mg/ml respectively. As for nicotine identification, the result of our study confirms findings from previous study [15], where the tested e-liquids labeled as having 6 mg/ml of nicotine resulted in none being identified after analysed conforming to nicotine labeling discrepancies among e-liquid products. Other than that, an inadequate handling or storage during manufacturing process of e-liquids can lead to oxidative degradation of nicotine to occur. In addition, other factors such as interaction with packaging materials and unstable formulation can also amplify nicotine degradation since addition of flavouring additives are known to affect e-liquid products stability [16]. Due to that, there should be a better quality control and manufacturing guidelines available globally in e-liquid production to ensure safety to the end users.

3.3 Identification of other Chemicals in e-Liquid

Several other chemical compounds to be concerned were able to be screened and identified in 7 out of 17 refill e-liquids (Table 2). RY4 had the most chemical compounds detected compared to only one types of chemical compound detected for other e-liquids. Octodrine which is a stimulant type of chemical compound commonly used in sport supplement was detected the most among other e-liquids. It is a central nervous stimulant that was initially developed as nasal decongestant to treat conditions like laryngitis and bronchitis. Hydrocodone, which is chemically identical to morphine, is a semisynthetic opioid used to treat acute and chronic pain by acting on opioid receptors resulting in euphoria feelings while testosterone propionate is an androgen and anabolic steroid (AAS) which is used initially for low testosterone level treatment in men and breast cancer before widely used for muscle mass building. As for cyclothiazide, it has been used in the treatment of hypertension as well as adjunctive therapy in edema. However, they are sometimes abused by people suffering from an eating disorder (i.e., bulimia nervosa), in order to lose weight. Meanwhile, methylthiouracil was first introduced as a thionamide anti-thyroid drug to treat hyperthyroidism but no longer in clinical use in most of the countries due to the higher rate of adverse reactions [17].

The presence of these chemical compounds might likely happen due to contaminants introduced during manufacturing process whereby other than nicotine, toxic substances can be intentionally mixed into the e-liquid for reasons not clearly understood. In addition, since e-cigarettes have proved to be an effective nicotine drug delivery system [18], the presence of the drugs or medication substances in e-liquid was believed due to the rapid onset of action of the system and inhalation has become increasingly common route of administration of illicit drugs. Highly additives substances such as amphetamines, codeine, oxycodone, methylphenidate, lysergic acid diethylamide (LSD), muscle relaxers and steroid as well as over the counter medication have all been used in e-cigarettes after being diluted in glycerine [19]. In a recent studies, a designer drug or a new psychoactive substance (NPS) was found in comercially available e-liquid which effects pharmacologically as a synthetic cannabinoid or cannabimimetic drug as well as methamphetamine was also successfully detected in the aerosol produced by e-cigarette system [20]. Moreover, other factors such as insufficiently pure compounds used as well as unforeseen reactions between chemical compounds during formulation can also be the reason [21].

Table 2
List of other chemical compounds identified in e-liquids

Name of drugs	Types of chemicals	E-liquids
Octodrine	Stimulant	TA, MLI, MS, ML, HB
Methylthiouracil	Anti-thyroid	RY4
Testosterone propionate	Androgen and anabolic steroid (AAS)	RY4
Hydrocodone	Opiate (narcotic) analgesics/opioid	RY4
Cyclothiazide	Thiazide diuretic	BM

4. Conclusion

Findings from this study showed that the locally sold e-liquid products in Malaysia contained a wide variety of flavour compounds as well as other chemical constituents that might possess unknown

health risk to the consumer. Thus, mandatory manufacturing standards for e-cigarette production especially refill e-liquid as well as laws as to be ruled out to control and act as safety measure to avoid any commercialisation of e-liquid products that is potentially unsafe to consume by the end users.

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