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Chemical Composition of E-liquid and Potential Health Implication in Electronic Cigarettes

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Abstract. Public interest in e-cigarettes in Indonesia has increased significantly in recent years. This was initially driven by claims that e-cigarettes as an alternative tool to help individuals quit conventional smoking. However, this product is increasingly attracting teenagers and people who did not previously smoke. This trend is largely due to the composition of e-liquids used in e-cigarettes, which often formulated with flavor compounds such as vanillin, menthol, and various fruit flavors that significantly enhance the sensory characteristics and consumer acceptability of the products. Many of these flavoring compounds are commonly used in food products with regulated limits. However, the toxicological effects of flavoring compounds in inhalation and the maximum exposure limit have not been comprehensively investigated. Moreover, the presence of nicotine as an addictive substance in e-cigarettes can encourage continuous use, consequently increasing the frequency and duration of exposure to flavoring compounds. These conditions raise concerns about potential health risks, particularly among youth who are frequently identified as active users. Therefore, this study aims to determine the concentration of vanillin, one of the most frequently detected flavoring compounds in e-liquids. The analyzed samples include vanilla, mango peach, *and* banana cream berry *flavor variants*. The concentration of vanillin was quantified using gas chromatography with Flame Ionization Detector (GC-FID). The analytical method was validated using several parameters, including precision, accuracy, linearity, LOD, and LOQ. The results showed that the concentration of vanillin detected in the three analyzed e-liquid samples were 8.36mg/mL, 2.04mg/mL, and 1.28mg/mL, respectively. The findings indicate that vanillin, commonly associated with sweet flavor profiles, was also identified in e-liquids labeled as fruit-flavored. These results highlight the widespread use of flavoring additives in e-cigarette products and emphasize the importance of monitoring their presence due to potential implications for public health.

Introduction

The use of e-cigarettes in Indonesia is increasing, which was initially driven by claims that e-cigarettes were developed as a tool to help people quit conventional smoking habits (Badan Pengawas Obat dan Makanan Republik Indonesia, 2017). However, during its development, this product has increasingly attracted teenagers and individuals who were not previously smokers, thereby expanding the target consumer beyond the initial target.

The rise of e-cigarette promotion has encouraged teenagers and non-smokers to try e-cigarettes, so the demand continues to increase. The main attraction of this product is the variety of flavors, such as vanillin, menthol, and some fruit flavors, which are food-grade compounds. Based on the Global Youth Tobacco Survey (GYTS), the prevalence of e-cigarette users increased from 0.3% in 2019 to 3% in 2021 (WHO et al., 2021). Meanwhile, data from the 2023 Health Policy Development Agency shows that the 10-14 year old age group is the largest group of e-cigarette users at 9.6%, followed by the 20-24 year old age group at 8.7% (Kementerian Kesehatan Republik Indonesia, 2023).

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E-cigarattes are devices that produce aerosols through the process of heating liquids, known as e-liquids. The e-liquid used in e-cigarettes consists of several main components, such as propylene glycol, glycerol, nicotine, natural and artificial flavors, and other additives (Goenka, 2025). Various flavor compounds are commonly formulated into e-liquids to produce specific aroma and taste characteristics, thereby increasing the sensory appeal of e-cigarettes (Krüsemann et al., 2021). However, during heating, the flavor compounds in e-liquid have the potential to undergo a pyrolysis process that produces oxidative radicals that can trigger the formation of additional toxic compounds, such as formaldehyde and other carbonyl compounds (Robertson et al., 2025). In addition, many manufacturers do not include the complete information about the e-liquid composition on the product label. Some studies have found discrepancies between the nicotine content or flavor listed on product labels and laboratory measurements, and even nicotine detected in some e-liquids labeled as nicotine-free (Badan Pengawas Obat dan Makanan Republik Indonesia, 2017). This condition shows the potential health risks and lack of regulatory transparency in the electronic cigarette industry.

Erna et al. conducted a study of 320 e-liquids, which were classified by categories (Krüsemann et al., 2020). There are 15 flavor categories, such as dessert, candy, fruit, coffee, sweets, and others. The study identified 18 chemical compounds, such as vanillin, ethyl butyrate, Cis-3-hexenol, Benzyl alcohol, and others. Among these compounds, vanillin was identified as the most common flavor compound found in various flavor categories. Based on research conducted by flavour chemicals from 30 categories of e-liquid flavors, showed that 42% of e-liquid samples contained vanillin flavor compounds. These results suggest that vanillin is widely used in e-liquid formulations. Most of the chemical compounds detected in e-liquids are listed in Generally Recognized as Safe (GRAS) by the FDA (Kassem et al., 2024). However, most studies supporting the determination of safe exposure limits for these chemical compounds under GRAS designation are primarily based on oral consumption. The safety of the chemicals on the GRAS list has not been adequately evaluated for inhalation exposure (Goenka, 2025). Vanillin is widely recognized as a food additive that is considered safe within established limits (BPOM, 2020). However, this does not necessarily imply that vanillin is safe when exposure occurs through inhalation.

Several flavor compounds in e-liquids have been studied to determine their safety for use in electronic cigarettes. According to research conducted by Serwood et. al., vanillin is one of the flavor compounds that can damage the airway epithelium (Sherwood & Boitano, 2016). The epithelium is a single cell layer that serves as an initial protector against inhaled particles, pollutants, and microorganisms and supports the exchange of oxygen and carbon dioxide. In addition, the epithelium can also detect pathogens and toxic substances. In addition, a previous study reported that vanillin caused harmful effects when vaporized (R et al., 2024). Continuous exposure to vanillin can irritate the epithelium, increase the risk of inflammation, and increase the likelihood of chronic diseases such as bronchitis and even cancer.

Currently, there are no regulations establishing a safe limit for flavor compounds in e-liquids (Badan Pengawas Obat dan Makanan Republik Indonesia, 2017), so the composition of flavor compounds in e-liquids is entirely dependent on the manufacturer's policy. This situation led many e-cigarette manufacturers to not include the complete composition on their product labels, so consumers unaware of how much flavor is contained in the product. The presence of nicotine as an addictive substance in e-cigarettes makes consumers tend to consume them continuously, so that exposure to vanillin will occur repeatedly and for a long time. These conditions raise concerns about potential health risks for the public, especially among youth who are frequently identified as active users. To overcome this issue, the analysis of vanillin content, as one of the most commonly found flavor compounds in various e-liquid categories (Omaiye et al., 2022), can be carried out using gas chromatography (GC) to obtain accurate data about the vanillin content in e-liquid. The results of this research will provide the scientific basis for developing regulations on the chemical composition of e-liquid products.

Experimental

Material and Methods

Analytical balance, volumetric flask (Iwaki Pyrex), Gas Chromatography – Flame Ionization Detector (GC-FID), vanillin (Fluka AG, Buchs SG), ethanol (Smart-Lab), and e-liquid samples (vanilla, mango peach, banana berry cream). The vanillin standard used is of food-grade quality (purity >99%).

Procedures

Sample Preparation

E-liquid products used as e-cigarette refillable liquids are obtained from young people who are known to be e-cigarette users. Three samples of e-liquid with mango peach, vanilla, and banana cream berry flavor variants were obtained for analysis. Samples are stored in their

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original packaging at room temperature. The analysis method used is laboratory experiments.

The analytical procedure was adapted from previously reported, 100mg sample e-liquid was weighed into a 10mL volumetric flask and dissolved using ACN as the solvent (Aszyk et al., 2018). However, in this study, ethanol was used as the solvent. The sample solution was dissolved to the boundary mark and homogenized before further analysis.

Preparation of Vanillin Standard Solution

A stock standard solution of vanillin was prepared at a concentration of 1mg/mL in ethanol.

Quantitative Analysis of Vanillin

Preliminary studies were conducted using e-liquid samples prepared with a standard mixture for method development. The obtained results indicated that the analytical procedure was suitable for the intended determination, therefore a comprehensive method validation was carried out to ensure the reliability of the method. The validation procedure evaluated several analytical parameters, including precision, accuracy, linearity, LOD, and LOQ.

Measured volumes of 1mL, 2mL, 3mL, 4mL, and 5mL of the standard solution were transferred into separate 10mL volumetric flasks and subsequently diluted with ethanol as the solvent.

Linearity

The linearity of the analytical method was evaluated using vanillin standard solutions prepared at six concentration levels of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500ppm. A calibration curve was developed by plotting the peak area against the concentration of the standard solutions. The linear regression equation will be used to analyze the relation between concentration and detector response, with the coefficient of determination (R^2) used as an indicator.

Precision

Precision was evaluated by analyzing a standard solution repeatedly under the same experimental conditions and the result were reported as relative standard deviation (RSD).

Accuracy

Accuracy was evaluated using spiked solutions at three concentration levels of 80%, 100%, 120%. Each concentration level was carried out in triplicate to ensure the reliability of the results. A vanillin standard addition was added to each sample at a concentration of 0.001mg/mL.

LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated using the standard deviation of the response and the slope of the regression line from the calibration curve.

GC Operational Conditions

Vanillin in e-liquid samples was analyzed using gas chromatography (GC). Component identification was performed by comparing the retention time of vanillin in the samples with that of the vanillin standard. The quantification of vanillin was carried out by comparing the peak area of the standard solution with the corresponding peak area detected in the sample chromatograms. The quantification analysis was performed using gas chromatography equipped with a capillary column (10-15m x 0.32 or 0.53mm long), 100% dimethyl-PSX (X-1) as the stationary phase with a film layer thickness of 1.5 to 3µm. The initial temperature was set at 135°C and held for 4 minutes, followed by a temperature increase to 200°C at a rate of 13°C/minute. Then the temperature was then further increased to 312°C at a rate of 6°C/minute, the final run time was 20 minutes. The injector temperature was maintained at 200°C. Gas chromatography use N_2 as the carrier gas at a constant flow rate of 25mL/min. The Gas Chromatography (GC) system was equipped with an *Flame Ionization Detector* (FID) and operated at 200°C. After the instrumental conditions were established, 1µL of both the sample and standard vanillin solutions were injected with a split ratio of 50:1. The analysis generated chromatograms indicating the detected compounds along with their retention times and corresponding peak areas.

Result and Discussion

The e-liquid sample analyzed in this study were selected from flavor variants frequently used by young consumers. Gas chromatography system was used to determine the concentration of vanillin in the e-liquid sample. The gas chromatography system was validated to ensure the reliability of the analytical performance.

The evaluated parameters included precision, calibration curve, accuracy, LOD and LOQ. The results of the method validation are represented in Table 1. Precision was evaluated by repeated injections of the vanillin standard solution, producing a relative standard deviation (RSD) of 0.6875%, indicating acceptable precision of the

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analytical method. The quantification analysis was carried out using curve calibration from six different concentration levels of the vanillin standard solution. Linearity was evaluated by plotting peak area (y-axis) and standard solution concentrations (x-axis). The calibration curve for the vanillin standard is presented in Figure 1. Linear regression analysis of the calibration data produced the linear equation y=1553.9x-3.5363. The coefficient of determination (R^2) was 0.9992, indicating a high linear correlation between the standard concentration and the GC-FID instrument response.

Table 1. Results of validation parameters.

Validation Parameters	Result	Acceptance Criteria (ICH) (Aszyk et al., 2018)
Linearity (R^2)	0,9992	R^2 > 0,999
Precision (RSD, n=6)	0,6875%	RSD < 2%
Accuracy	91% - 115%	80% - 120%
LOD	0,0239 mg/mL	-
LOQ	0,0799 mg/mL	-

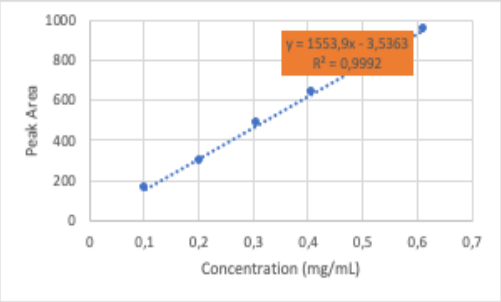
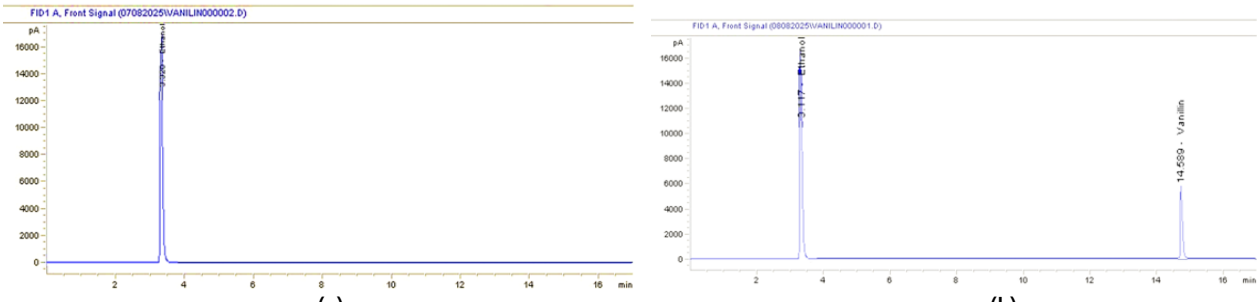


Figure 1. Vanillin standard calibration curve between concentration and peak.

The limits of detection (LOD) and limits of quantification (LOQ) obtained were 0.0239mg/mL and 0.0799mg/mL, respectively (Nabila et al., 2025). These results show that the analytical method possesses adequate sensitivity for detecting vanillin at very low concentrations in e-liquid samples. The accuracy of the method was evaluated by analyzing e-liquid samples spiked with vanillin standard at three different concentration levels. The recovery values varied between 91% to 115%, indicating acceptable analytical accuracy. Based on the calibration curve, precision, LOD, LOQ, and accuracy parameters, the developed method can be considered reliable and suitable for the determination of vanillin in e-liquid samples using GC-FID.

The sample preparation procedure in this study was adapted from the analytical method carried out by Aszyk et al., which employed ACN as the solvent (Aszyk et al., 2018). In this study, ethanol was selected as the solvent. The selection of ethanol was mainly based on laboratory safety considerations as ethanol is generally considered less hazardous than CAN during routine analytical. In addition, ethanol has the ability to dissolve compounds with varying degrees of polarity, enabling effective dissolution of the components present in the e-liquids samples during the preparation process (Mao et al., 2020). Moreover, ethanol is compatible with gas chromatographic analysis due to its volatility and minimal interference with GC-FID detection.

Figure 2 illustrates the chromatograms obtained from the GC-FID analysis of the chemical content in the e-liquid. Figure 2(a) shows the chromatogram of the ethanol used as the solvent. Figure 2(b) shows the chromatogram of the solution containing the vanillin standard, which was prepared as a reference for identifying the presence of vanillin in the sample. The peak observed at retention time between the 3rd and 4th minutes is identified as ethanol and the peak that appears between the 14th and 15th minutes is identified as vanillin. This chromatogram also shows the corresponding peak areas for each detected compound, which were subsequently used for quantitative evaluation of vanillin in the e-liquid samples.



(a) (b)

Figure 2. GC-FID chromatograms of: (a) Ethanol; (b) Vanillin standard.

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Figure 3 illustrates the chromatogram of one representative e-liquid samples, representing main components identified in this study. The analytical results indicated the presence of vanillin in the e-liquid samples.

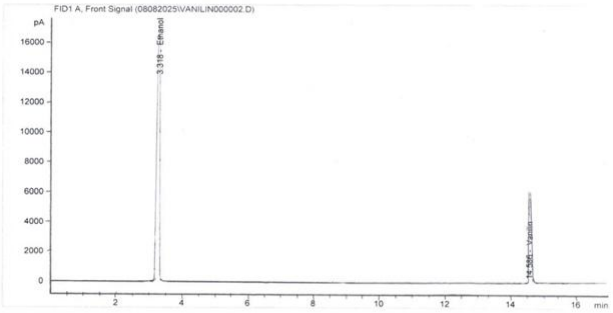

Figure 3. GC-FID chromatograms of e-liquids samples.

However, the analysis indicated considerable variability in composition among different e-liquids brands, as represented in Table 2.

Table 2. Chromatographic data for vanillin analysis.

E-liquid Variant	Retention Time	Peak Area	Vanillin content (mg/mL)
Vanilla	14,579	21,8223	8,36
Mango Peach	14,580	5,2852	2,04
Banana Cream Berry	14,586	3,4164	1,28

The analysis of three e-liquid samples showed that vanillin was detected in all samples with different concentrations. Table 2 shows that the vanilla variant indicated the highest vanillin concentration 8.36mg/mL, which was greater than previously reported that was 6,1mg/mL (Tierney et al., 2016). The mango peach and banana cream berry variant had vanillin concentrations of 2.04 and 1.28mg/mL, respectively. This is further supported by a previous study, which indicates that vanillin was predominantly identified in e-liquids with sweet, powerful, creamy, vanilla-like flavour variants (Krüsemann et al., 2020). However, the results of this study showed that vanillin was also detected in the fruit-flavored e-liquid variants. This discrepancy can be explained by variations in flavoring formulation practices among e-liquid manufacturers.

Vanilin (4-hydroxy-3-methoxybenzaldehydehyde) is an aromatic compound characterized by the presence of aldehyde (-CHO), hydroxy (-OH) and methoxy ($-OCH_3$) functional groups attached to a benzene ring, which causes vanillin to have semi-volatile properties. Accordingly, this study employed GC-FID method to determine the quantity of vanillin in e-liquid samples. The analytical principle is based on the application of heat during GC-FID analysis. In gas chromatography, the e-liquid sample is vaporized at controlled temperature. The volatile compounds are separated within the chromatographic column based on their interactions with the stationary phase and differences in volatility before detection by the Flame Ionization Detector (FID). This analytical approach is relevant to the operational mechanism of electronic cigarettes, in which e-liquids are heated, leading to volatilization of its components and form aerosols that can be inhaled by the user.

Vanilin also has the potential to undergo thermal degradation producing aldehyde and carbonyl derivatives compounds. Continuously inhalation may lead to the accumulation of vanillin and its degradation products within the respiratory tract, potentially inducing oxidative stress in the pulmonary epithelial tissue. Furthermore, long-term exposure to carbonyl compounds can also increase the formation of reactive oxygen species (ROS), which may potential to cellular damage. Although vanillin is widely used as a flavoring agent, repeated exposure over long periods of time could increase the risk of respiratory irritation (Jabba et al., 2021).

According to a previous study, the recommended daily exposure limit of vanillin is approximately 67mg/day (Tierney et al., 2016). The average consumption of e-liquid by e-cigarette users is estimated at 5mL per day. From the results of this study, vanillin content in the e-liquid samples was determined reach 8.36mg/mL. Assuming a daily e-liquid intake of up to 5mL, the estimated exposure to vanillin would reach 41.8mg/day. This study indicates that the vanillin exposure from the analyzed e-liquid samples remains below the recommended limits, according to Tierney et al. However, considering that many commercial e-liquid products do not report complete chemical compositions, it is necessary to establish regulatory frameworks that require manufacturers to provide a complete profiles of all chemical compounds and define permissible limits for the concentration of each compound in e-liquid products.

Conclusion

The presence study identified the presence of the flavouring compound vanillin in all three e-liquid samples, with the highest concentration detected in the vanilla-flavoured e-liquid variants. Furthermore, the flavoring

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compounds contained in e-liquid are known to have specific recommended exposure limits to ensure user safety. This study indicates that the vanillin concentration in the analyzed samples remains within the recommended limit. Currently, Indonesia has not established exposure limits for flavoring compounds in e-liquid. The quantified concentration of vanillin in this study provides an evidence-based reference for the development of regulatory guidelines on limited flavouring compositions in e-liquids, as an effort to reduce the attractiveness of e-liquids, especially for adolescent users.

Furthermore, this study developed a GC-FID method for the quantitative analysis of vanillin flavor compounds in e-liquid. The method can be applied in laboratories to monitor the composition of flavoring compounds in e-liquids. Further research is needed to characterize and profile other flavoring compounds across diverse e-liquid formulations.

Conflict of Interest

The authors declare that there is no conflict of interest

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