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AUTHENTICATION OF CLOVE LEAF OIL IN PRODUCTS (*Syzygium aromaticum* (L.) Merr. & L. M. Perry) USING GC-MS AND FTIR METHODS COMBINED WITH CHEMOMETRIC

Pengesahan Minyak Daun Cengkih (*Syzygium aromaticum* (L.) Merr. & L. M. Perry) dalam Produk Menggunakan Kaedah GC-MS dan FTIR Yang Digabungkan dengan Kemometri

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Abstract

The production cost and market demand for clove oil are significantly high due to its great commercial value and benefits. However, the extraction yield of clove oil is very low at around 1%, causing the act of adulteration of essential oils to reduce production costs. Therefore, this study aims to determine the addition of adulterants in essential oils using the authentication technique. The samples used are clove oil from dry leaf distillation, products A, B, and C on the market. The GC-MS and FTIR methods combined with PCA (Principal Component Analysis) and PLS (Partial Least Square) multivariate calibration analysis were selected in the authentication to detect clove oil adulteration. The constituent's presence in distilled clove oil from GC-MS analysis was eugenol (49.63%), β-Caryophyllene (28.25%), alpha-Humulene (8.92%), alpha-Copaene (2.15%), delta-Cadinene (1.61%), and Caryophyllene oxide (1.50%). Six concentrations were prepared for FT-IR analysis of clove leaf oil and turpentine oil mixture, which was estimated by PLS and PCA chemometrics. Turpentine oil was used as counterfeit, which was usually added to clove oil products. The PLS analysis of FTIR obtained optimized wavenumbers, 2960-2860 cm^{-1}. The equation y=0.9998x+0.0096 had an R^2 value of 0.9998, as well as RMSEC, RMSECV, and RMSEP values of 0.22%, 0.76%, and 1.20%, respectively. The PCA analysis can categorize the oil based on the main component types of distilled clove leaf oil, turpentine oil, and market oil, namely products A, B, and C. The results showed that product A oil was in the same quadrant as distilled clove leaf oil. Moreover, no clove leaf oil product had similar physical and chemical characteristics to turpentine oil.

Keywords: authentication, clove leaf oil, Fourier transform infrared, gas chromatography-mass spectrometry, turpentine oil

Abstrak

Minyak cengkih mempunyai nilai dan faedah komersial yang besar, pada masa ini permintaan untuk ketersediaan minyak cengkih di pasaran adalah sangat tinggi namun, hasil perahan minyak cengkih adalah sangat rendah, iaitu sekitar 1% dan mengakibatkan kos pengeluaran minyak cengkih yang agak tinggi. Ini menyebabkan pemalsuan minyak pati untuk mengurangkan kos pengeluaran

dan mengekalkan jumlah minyak pati. Satu teknik yang boleh digunakan untuk mengesan kehadiran bahan tiruan dalam minyak pati adalah melalui pengesahan. Kaedah GC-MS dan FTIR digabungkan dengan analisis penentukuran multivariat PCA (analisis prinsip utama) dan PLS (*partial least square*) telah dipilih dengan tujuan untuk mengesan tindakan pemalsuan pada minyak cengkih. Kandungan minyak daun cengkih suling daripada hasil analisis GC-MS iaitu eugenol 49.63% β-Caryophyllene 28.25%, alpha-Humulene 8.92%, alpha-Copaene 2.15%, delta-Cadiene 1.61% dan Caryophyllene oksida 1.50%. Analisis FT-IR dijalankan dengan membuat 6 siri kepekatan campuran minyak daun cengkih dan minyak turpentin dan kemudiannya dianalisis dengan kemometrik PLS dan PCA. Keputusan analisis PLS daripada FTIR menunjukkan bahawa nombor gelombang yang dioptimumkan ialah 2960-2860 cm^{-1}. Persamaan yang terhasil ialah y = 0.9998x + 0.0096 dengan nilai R^2 0.9998; Nilai RMSEC (0.22%; nilai RMSECV 0.76%; dan nilai RMSEP 1.20%. Hasil analisis PCA mampu mengklasifikasikan minyak berdasarkan jenis komponen utama minyak suling daun cengkih, minyak turpentin, dan minyak daun di pasaran (A, B, dan C), di mana minyak produk A berada dalam kuadran yang sama dengan minyak daun cengkih suling dan tiada produk minyak daun cengkih yang mempunyai ciri fizikal dan kimia yang serupa dengan minyak turpentin.

Kata kunci: pengesahan, inframerah transformasi Fourier, kromatografi gas-spektometri jisim, minyak daun cengkih, minyak terpentin

Introduction

Clove (*Syzygium aromaticum*) is a spices that has long been used in medicine and as a food preservative [1]. This plant is a natural source that contains many secondary metabolite compounds, namely phenolics such as eugenol, eugenol acetate, and gallic acid. Therefore, its leaf oil, namely clove oil, has great potential for applications in the pharmaceutical, cosmetic, food, and agricultural fields [2]. The main constituents in clove flower buds, stalks, and leaves are eugenol and β-caryophyllene [3]. Meanwhile, the essential oil content in clove leaves is 58.6% eugenol, 21.4% β-caryophyllene, 5.6% α-humulene, and 2.9% caryophyllene oxide [4].

Clove oil can be produced from flowers and stems, including leaves that have the highest economic value. The price range from various parts is not significantly different, making it a widely traded product. This makes the leaves more economically valued for their potential to be processed into oil [5].Currently, the market demand for clove is very high due to its great commercial value and benefits. However, the extraction yield is significantly low at around 1%, leading to a high cost of production. This has consequently caused the adulteration act of essential oils to reduce production costs [6]. According to Hong et al. [7], essential oil adulteration is a major problem in today's global market by mixing cheaper components of lower quality in high-value essential oils.

Detection of clove oil adulteration is carried out to ensure that the oil sold in the market meets the established quality standards. Meanwhile, a frequently used type of counterfeiting material is the addition of turpentine oil with the main component which can cause an increase in the α-pinene content. This will reduce the specific gravity and solubility value of the oil in alcohol and affect the odor [8]. Turpentine oil is widely selected as an adulterant in essential oils because it is relatively cheap, with a lighter and less polar fraction is lighter [9]. The technique that can be performed to detect the addition of counterfeiters in essential oils is authentication. This technique is conducted using GC-MS and FTIR methods, which will be analyzed with PCA (principal component analysis) and PLS (partial least square) multivariate calibration to detect counterfeiting of clove oil [10].

The technique commonly used for essential oil quality control is gas chromatography (GC) coupled with mass spectrometry (MS). This is because the detection of volatile compounds in essential oils can quickly, easily, and accurately be identified using the technique. In recent years, spectroscopic techniques with a PCA chemometrics approach have often been proposed as an alternative to chromatographic methods in detecting the adulteration of essential oils [11].

Another method commonly used is Fourier transforms infrared (FTIR) combined with multivariate calibration chemometrics PLS and PCA to detect specific functional group absorption [12]. The authentication of essential oils using the FTIR is carried out in the fingerprint area,

which is the characteristic of a compound with the spectrum and sample concentration entered in a one-step model [13]. In this study, the profile analysis of the chemical components of clove oil using GC-MS and FTIR methods was will be analyzed by multivariate calibration of PCA and PLS to authenticate clove oil products in the market.

Materials and Methods

Materials

The material used was dry clove leaves harvested in June 2022 from Green Herbal Yogyakarta Company. Fresh clove leaves were determined at the Ahmad Dahlan University Biology Laboratory by sending fresh whole samples, including leaves and stem, for accurate identification. Subsequently, the clove oil brands A, B, and C as samples were obtained from e-commerce (Shopee). Turpentine oil was obtained from PT. Syailendra Bumi Investama.

Tools

The tools used were a set of distillation tools, beakers, measuring pipettes, micropipettes, GC-MS Agilent 7890A instrument with specifications, capillary column type HP-5DB MSDB 5DB-5 (60 m x 250µm x 0.25µm), carrier gas used helium, injector temperature 250 °C with an injection volume of 0.2 µL, split ratio 1:200 with a split speed of 50 mL/minute and the initial temperature of 50 °C held for 2 minutes. The rate of increase is as follows 10 °C/minute to 99 °C, 2 °C/minute to 225 °C held for 20 minutes, and 5 °C/minute to 250 °C. The final temperature reached 250 °C. The detector used was Agilent 5975C MS with a mass range of 35-550.

The FTIR instrument used Thermo Scientific Nicolet iS10 with beam splitter using KBr and deuterated triglycine sulfate (DTGS) detector. The readings were taken at wavenumbers: 4000-650 cm^{-1}, resolution 8, and 32 scans.

Procedure: Clove leaf preparation

A 3 kg dried clove leaves obtained from Green Herbal Yogyakarta Company were dry-sorted, and the sizes were reduced by 2-3 cm.

Authentication method: Analysis using GC-MS with PCA chemometrics

Samples of clove oil, turpentine oil, and market products were put into 2 mL vials and placed sequentially in the sample container of the GC-MS device. The n-hexane solvent was also placed in a 2 mL vial in the last container. A total of 0.2 µL oil samples were automatically injected into the GC-MS using a syringe. After the injecting one sample, the syringe automatically washes and perform column cleaning. At the column cleaning stage, the GC-MS was run without inserting any sample. This process was repeated three times, and the samples were identified using MS. Subsequently, data were analyzed on GC-MS in the form of the relative area obtained from chromatogram results and m/z fragmentation of compounds.

Sample calibration and validation for FTIR with PCA and PLS chemometrics

A total of 7 concentrations was prepared for each oil in the form of distilled clove leaf oil (MC) 100%v/v, turpentine oil (MT) 100%v/v. Further concentration series (2 mL) were also prepared, namely MC:MT (90:10), (80:20), (70:30), (60:40), and (50:50) %v/v for calibration and validation. This was followed by the analysis of clove leaf oil, turpentine oil, as well as samples A, B, and C using an FTIR [12].

Data analysis: GC-MS data analysis of PCA chemometrics combination

GC-MS data in the relative area was collected by grouping the essential oil components with clove oil products on the market brands A, B, and C using PCA followed by biplot with MINITAB 20 software. This grouping was based on data diversity, which produces scree, score, loading plots, and biplot graphs [14].

FTIR data analysis of combined PLS and PCA chemometrics

The FTIR data were analyzed by multivariate calibration in MINITAB 20 program using PCA and PLS techniques for quantification. The original spectrum absorbance data, the spectrum with a preliminary process involving all absorption features, and part of the absorption data were processed according to the spectrum segmentation area. Subsequently, the

calculation of the RMSE value was obtained by squaring the subtraction of the actual value with the predicted value summed and divided by the number of data [15].

Results and Discussion

Component analysis of clove leaf oil

The analysis of clove leaf oil using the GC-MS method was applied to determine the oil component and the percentage of essential oil compound content. The method had a high sensitivity to quantitatively and qualitatively volatile compounds. Furthermore, it was a fast and accurate method for separating complex mixtures and producing data on the structure as well as the identity of organic compounds. In GC-MS, the AUC produced by the chromatogram was directly proportional to the concentration of the components contained in the sample. The number of essential oil components and their percentage levels were obtained from analysis with GC. This was followed by using MS to determine the type of compounds contained in clove leaf oil using spectra derived from NIST and WILEY libraries. Meanwhile, the chromatogram of GC separation results of clove leaf essential oil is presented in Figure 1.

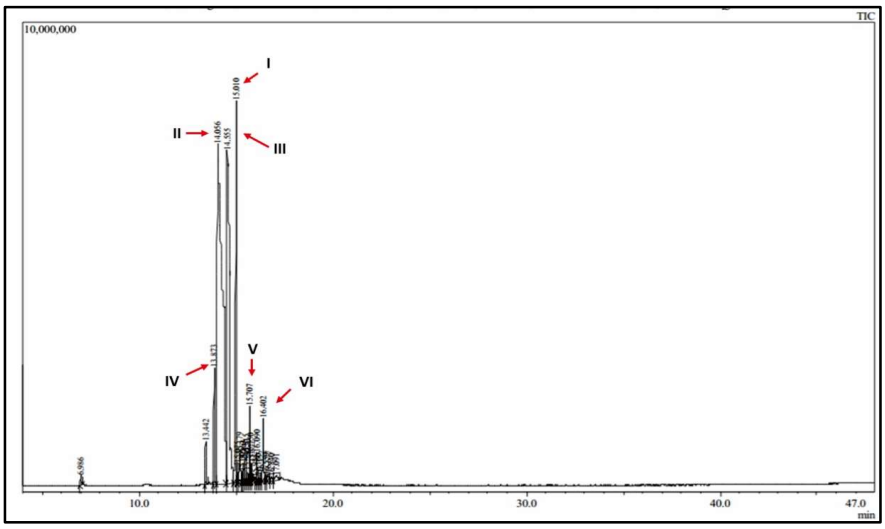

Figure 1. GC Chromatogram for clove leaf oil

Based on the chromatogram results of clove leaf oil in Figure 1, 25 chromatogram peaks were obtained, which showed the profile of the oil components. Subsequently, 6 main peaks were selected, each of which was estimated to contain eugenol (3-Allyl-6-methoxyphenol), trans-caryophyllene, α-humulene, α-copaene, δ-cadinene, and caryophyllene oxide. The component analysis of clove leaf essential oil was also replicated 3 times, and the results are presented in Table 1.

The results of the analysis using GC-MS method presented in Table 2 obtained the constituent components percentage of clove leaf oil, including eugenol (3-Allyl-6-methoxyphenol) (49.63±0.20) > trans-caryophyllene (28.25±0.22) > α-humulene (8.92±0.09) > α-copaene (2.15±0.04) > δ-cadinene (1.61±0.27) > caryophyllene oxide (1.50±0.05). There were also varying CV (Coefficient of Variation) values, with the smallest value of 0.40 in the eugenol compound. Meanwhile, the largest CV value of 16.92 was found in the δ-cadinene compound, which was caused by the large deviation between replications.

Analysis of clove leaf oil using GC-MS produced a retention time (tR) based on the separation results from GC. The mass spectra analysis was carried out using the base peak with the highest frequency in the spectrum, namely 100%, and Similarity Index (SI). Furthermore, to determine the closeness of the chemical structure of the essential oil components, the standard target was compared with the compound components in the spectra

by comparing the spectra of the NIST and WILEY 9.LIB libraries in the GC-MS software [16]. The detected compound was similar to the comparison data when the SI value approached 100% or above 90%. The analysis results in Table 2 showed that the detected clove leaf oil components had an SI value of >90%. Therefore, it can be concluded that the components of clove leaves were appropriate to the comparator spectra.

Table 1. The analysis results on the essential oil components of clove leave with GC-MS

No.	tR (minutes)	% Area	% Area±SD	CV (%)	SI	BM	Compound
I	14.056	49.49	49.63±0.20	0.40	97	164	eugenol
	14.058	49.54					
	14.080	49.86					
II	14.555	28.08	28.25±0.22	0.78	97	204	trans-caryophyllene
	14.555	28.17					
	14.556	28.50					
III	15.011	9.01	8.92±0.09	1.01	97	204	α-humulene
	15.011	8.91					
	15.011	8.83					
IV	13.873	2.19	2.15±0.04	1.68	95	204	α-copaene
	13.874	2.14					
	13.872	2.12					
V	15.707	1.30	1.61±0.27	16.92	95	204	δ-cadinene
	15.706	1.80					
	15.706	1.74					
VI	16.402	1.55	1.50±0.05	3.00	96	220	caryophyllene oxide
	16.404	1.50					
	16.403	1.46					

Note: SI = Similarity Index, MW = Molecular Weight

Table 2. The analysis results on the main components of clove leaf oil, turpentine oil, product A, B, and C

Oil Components	Percentage(%)				
	Clove Leaf Oil	Product A	Product B	Product C	Turpentine Oil
Eugenol	49.63±0.20	49.81±2.79	21.92±0.20	28.32±0.12	N/A
β-caryophyllene	28.5±0.12	32.49±0.14	19.97±0.12	12.83±0.02	N/A
α-humulene	8.92±0.09	10.43±0.03	10.03±0.05	3.13±0.45	N/A
α-copaene	2.16±0.04	1.58±0.03	1.71±0.07	0.57±0.01	N/A
δ-cadinene	1.61±0.27	0.53±0.02	3.30±0.02	1.21±0.26	N/A
Caryophyllene oxide	1.50±0.05	2.16±0.03	3.89±0.03	1.31±0.08	N/A
α-pinene	N/A	N/A	0.53±0.06	7.32±0.06	55.30±0.61
δ-carene	N/A	N/A	N/A	23.19±2.47	19.49±0.22
β-pinene	N/A	N/A	0.33±0.13	1.79±0.12	3.56±0.06
Camphene	N/A	N/A	N/A	N/A	2.533±0.17

Description; N/A= Not detected

Component profiles comparison of clove leaf oil, turpentine oil, and other clove leaf oil products in the market (A, B, and C)

This study used three types of clove leaf oil products from e-commerce (online shops) because the manufacturers were more varied. The selection of clove leaf oil products was based on predetermined criteria, namely clove leaf oil that was 100% pure from leaves and without mixture, within a price range of IDR 20,000-35,000, purchased by at least 100 buyers, and having a good store rating.

One type of clove leaf oil product had a certificate of analysis (CoA), namely product A. The CoA will show that the product has good quality and meets predetermined quality standards. This guaranteed the purity of the product and ensured no adulterants such as turpentine oil was contained. Meanwhile, products B and C do not have CoA and were suspected of adding adulterated oil because the price was very cheap with a large enough volume.

In this study, adulterated turpentine oil was obtained from Ddistillers (Syailendra Bumi Investama Company). a Central Java which has a CoA. Generally, turpentine oil is usually used as an adulterated oil because the price is relatively cheap, with a lighter and less polar fraction. However, the addition of turpentine oil can cause changes in the physicochemical properties of clove leaf oil, such as refractive index and specific gravity. This can also reduce the number of chemical components in the oil, thereby influencing the aroma of the oil. No derivatization was carried out in the analysis stage using GC-MS because the sample was a volatile compound.

Table 3. Functional group prediction results and vibration model of clove leaf oil and turpentine oil

Tape	Wavenumber (cm^{-1})			Functional Groups	Vibration Models	Intensity
	Clove Oil	Turpentine Oil	Reference [17, 18]			
1	3522	-	3000-3600	O-H (alcohol, acid, H bonding)	Hold out	Medium
2	2925	2953	3000-2840	C-H	Hold out	Strong
3	1637	1640	1667	C=C (para aromatic)	Buckling	Weak
4	1511	-	1600 and 1475; 1511 (eugenol acetate)	C=C (aromatic)	Hold out	Medium
5	1461	1462	1469	Methylene ($-CH_2-$)	Buckling	Medium
6	1451	1449	1446	Methyl ($-CH_3$)	Buckling	Medium
7	1265, 1231, 1204, 1181, 1147, and 1121	-	1300-1000	C-O (ether)	Hold out	Strong

The results presented in Table 3 showed that the largest component of clove leaf oil was eugenol. In the distilled clove leaf oil, eugenol was 49.63±0.20 > Product A (49.81±2.79) > Product C (28.32±0.12) > Product B

(21.92±0.20). Meanwhile, in the β-Caryophyllene compound, the content was Product A (32.48±0.14) > clove leaf oil (28.253±0.22) > Product B (19.97±0.12) > Product C (12.83±0.02). Eugenol is a phenolic compound and the largest component in clove oil, with a distinctive color and aroma in clove leaf oil, which can turn brown when in contact with air due to oxidation events. The previous study by Hasim et al. [4] related to the isolation of compound content in clove leaves showed that the eugenol component (58.6%) and β-Caryophyllene (21.4%) were obtained. Meanwhile, according to the Indonesian National Standard 06-2387-2006, the requirement for eugenol content in clove leaf oil is at least 78%, and β-Caryophyllene is a maximum of 17%. The concentration of eugenol and β-Caryophyllene levels can be different due to several factors, namely variations between seasons, soil conditions, growing areas, cultivation and harvesting processes of clove leaves, as well as distillation processes [19]. The component analysis of clove leaf oil, turpentine oil, as well as products A, B, and C, are shown in Table 2.

The alpha-pinene compound was turpentine oil's largest component (55.30±0.61), detected in products B and C with values of 0.53±0.06 and 7.32±0.06, respectively. The result of the component analysis in product B detected beta-pinene compound of 0.33±0.13, while C found delta-carene 23.19±2.47 and beta-pinene 1.793±0.12. Meanwhile, products B and C may contain adulterants, namely turpentine oil. Bhuiyan [20] analyzed compound components in clove leaf oil *Syzygium caryophyllatum* (L.) Alston species by GC-MS method and detected alpha-pinene compounds of 0.33% and beta-pinene of 0.45%. According to Oboh et al. [21], in a study related to the components in clove oil, the GC-MS method found alpha-pinene compounds and beta-pinene with values of 13.09% and 45.44% in *Eugenia aromatica Kuntze* species.

PCA of clove leaf oil with turpentine oil and market products

The PCA analysis was carried out by entering the AUC of pure clove oil components, the AUC of turpentine oil, pure clove leaf oil, as well as the AUC of products A, B, and C. This process was replicated three times to determine whether there were affecting factors. The location of the quadrants showed the similarity between the physicochemical properties of the constituent components of clove leaf oil.

PCA analysis was conducted using MINITAB 20 software, the latest version of MINITAB. The result of the analysis was a principal component (PC) that represented a large amount of variation in the initial data, where PC1 contained the most variance. The output of the PCA analysis of the component profiles of clove leaf oil, turpentine oil, as well as products A, B, and C are shown in Figure 2.

PCA analysis showed that each PC produced eigenvalue, proportion, and cumulative. The eigenvalue describes the variation explained by the data for each PC and the magnitude of the how much the variables on the formation of the matrix properties [22]. Proportion described the data variation for each influential PC with the number of original data variables. Meanwhile, the cumulative value describes the cumulative amount of proportion.

Figure 2 shows that the 16 PC generated corresponded to the number of variable data entered. The analysis results indicated that PCA can reduce the initial data with 16 PC variables and explained by 2 new variables (PC1 and PC2) to provide data information by 97%. This is revealed from the eigenvalue >1, close to zero, without effect, and was not correlated [23, 24]. Meanwhile, the score plot of the chemical components of clove oil, turpentine oil, and product oil is shown in Figure 3.

The score plot in PCA described a plot of two PC that provided information regarding the patterns present in the sample. The plot of the initial PC usually had the most variation in the data. The score plot displayed in this study was divided into 4 quadrants, where the plots of turpentine oil and products B and C oil were in different quadrants. Meanwhile, clove leaf oil and product A were in the same quadrant. This indicated that the closer the distance between plots, the closer the relationship among the samples analyzed [25].

Based on Figure 2, the main component of turpentine oil was in a different quadrant, located separately from the other oils analyzed. The product oil showed that the main components are not similar to the adulterated sample's. Meanwhile, product A and clove leaf oil are in the same quadrant, indicating that both samples had similar physical and chemical properties. Products B and C were in different quadrants from clove leaf oil but not with turpentine oil due to the presence of impurities or other oil mixtures. The results showed that the PCA chemometric method can be used to analyze or classify clove leaf oil with adulterants and products in the market.

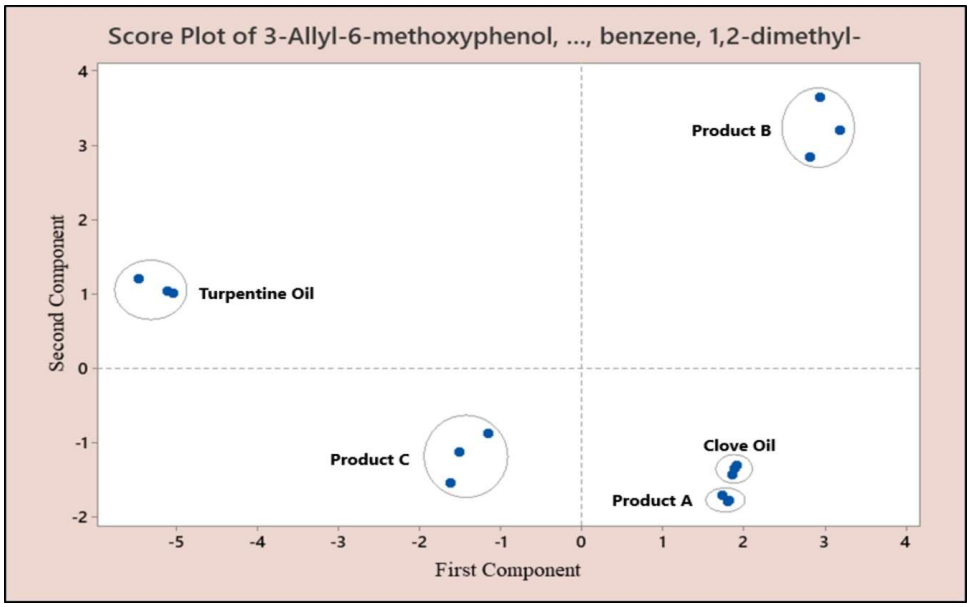

Figure 2. PCA plot scores from GC-MS of clove leaf oil, turpentine oil, product A, product B, and product C

Profile analysis of clove leaf essential oil by FTIR spectroscopy

The stages of analyzing the essential oil profile of clove leaves with the FTIR spectroscopy method were conducted using the functional groups. This was followed by the quantitative analysis with PLS and PCA.

Analysis of clove leaf essential oil by FTIR spectroscopy

FTIR spectroscopy is a widely used technique for essential oil authentication. This is because FTIR spectrophotometry can measure vibrations of functional groups as a consequence of electromagnetic radiation with the sample to produce a spectrum with fingerprinting properties [26]. Essential oils are volatile compounds consisting of a complex system of homogeneous mixtures of various compounds, and each species contains more than 100 constituents. Furthermore, essential oil fingerprint analysis can be used to distinguish species and chemotypes (common components) of different plants, representing a qualitative approach to the authentication and quality evaluation of essential oils [27, 28].

FTIR spectra can be distinguished from other compounds or samples based on wavenumber position, intensity, and the number of peaks [28]. The spectral analysis results of clove leaf oil and turpentine oil at wavenumbers between 4000 and 400 cm^{-1} are shown in Figure 3. The axis (x) is the wavenumber indicating the functional group, and the axis (y) is the absorption intensity [29]. The spectra analysis results showed that the infrared absorption peaks of both samples were at various wavenumbers. Further, Table 3 presented the predicted functional groups and vibrational models of clove leaf oil and turpentine oil.

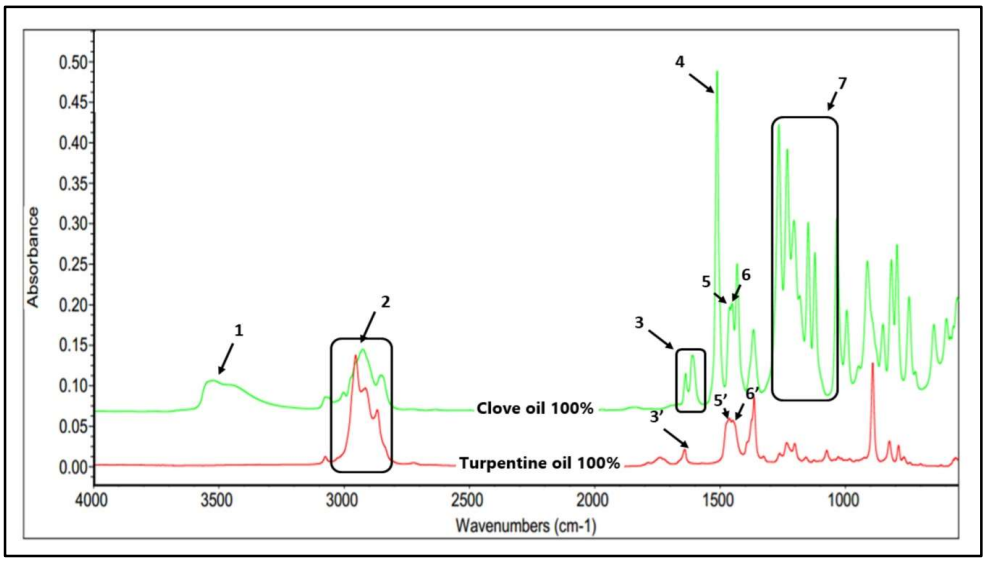

Figure 3. FTIR spectra of clove leaf oil and turpentine oil at wavenumbers 4000-600 cm^{-1}

The analysis results of the clove leaf oil functional groups in Table 3 showed a peak at wavenumber 3522 cm^{-1}, which was the O-H Phenol group with stretching vibrations. O-H groups typically at wavenumbers 3600-3000 cm^{-1}, as stated by Yang et al. [30] related to the analysis of eugenol compounds using FTIR instruments at wavenumbers obtained 3417 cm^{-1} with stretching vibrations. At wavenumber 2925 cm^{-1}, there was a C-H alkane functional group with stretching vibrations. Meanwhile, in turpentine oil at wavenumber 2953 cm^{-1}, the C-H alkane group will appear at 3000-2850 cm^{-1} [17]. At wavenumber 1511 cm^{-1}, an aromatic C=C functional group with the usual stretching vibrations. This indicated the presence of aromatic groups such as benzene in eugenol compounds, which were the main components of clove oil. The absorbance results in this study are 1637 cm^{-1} for the clove oil and 1640 cm^{-1} for turpentine oil, which is the C=C stretching vibration of the aromatic group [17]. Eugenol showed distinctive sharp peaks between 1640-1500 cm^{-1} of C=C aromatic because the functional groups of eugenols included double bonds. Therefore, these wavenumbers can provide unique spectral information with significant contributions to substance identification [31, 32, 33]. The FTIR spectra of clove oil and turpentine oil are shown in Figure 3.

In turpentine oil, the wavenumber 1640 cm^{-1} appeared, indicating the C=C alkene functional group with stretching vibrations, which showed the alkene group in the -pinene compound [34]. At 1461 cm^{-1} in clove oil and 1462 cm^{-1} in turpentine oil, there was a functional group (-CH_2-) with bending vibrations that usually appeared around the wavenumber 1465 cm^{-1} [17]. Furthermore, at wavenumbers 1451 cm^{-1} from clove, as well as 1449 cm^{-1} and 1363 cm^{-1} from turpentine oil, there was a bending vibration of the methyl group (-CH_3) [17]. The wavenumber of 1363 cm^{-1} in turpentine oil can indicate that α-pinena contains a gem-dimethyl group (C-(CH_3)$_2$) [34]. In clove leaf oil, wavenumbers 1265, 1231, 1204, 1181, 1147, and 1121 cm^{-1} contain C-O ether groups with stretching vibrations, which often appear at 1300-1000 cm^{-1} [17]. This wavenumber also indicated the presence of an ether group in the eugenol compound, such as C-O ether that appears at 1098 cm^{-1} [30]. The wavenumber 1300-1000 cm^{-1} not only described the C-O functional group but also explained the presence of CH_2 deformation vibrations in eugenol and eugenol acetate, besides C–C strain and C–H bending vibrations [18].

Based on the spectrum produced from clove leaf oil, the functional groups of the constituent components of clove leaf oil are eugenol and turpentine oil which contain α-pinene compounds. The wavenumber areas 1560-1480 cm^{-1}, 1814-1700 cm^{-1}, and 2954-2780 cm^{-1}

in clove leaf oil can be used in the PLS model developed for quantifying total eugenol [33]. The concentration series between clove oil and turpentine oil is presented in Figure 4. The peaks showed a variation in peak intensity in the difference in the concentration of clove oil and turpentine oil. Meanwhile, the variation in peak intensity during FTIR analysis indicates an effect that occurs when mixing clove leaf oil and turpentine oil.

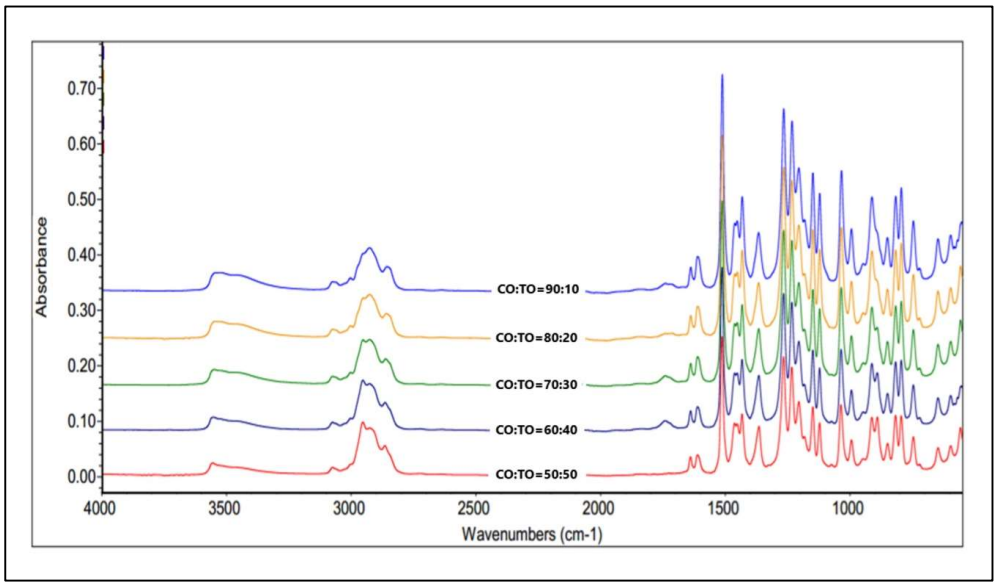

Figure 4. FTIR spectra of concentration series on clove leaf oil and turpentine oil at wavenumbers 4000-600 cm^{-1}

The absorbance results at each concentration ratio were used in the PLS and PCA chemometrics analysis. The FTIR spectra of clove leaf oil products A, B, and C circulating in the market are presented in Figure 5. Oil products were directly analyzed in the form of absorbance data using FTIR at wavenumbers 4000-400 cm^{-1}. The peak number of clove oil waves on the market is similar to that of the refined clove oil. However, there were differences in peaks indicating the OH functional group, namely products B and C, as shown in Table 4.

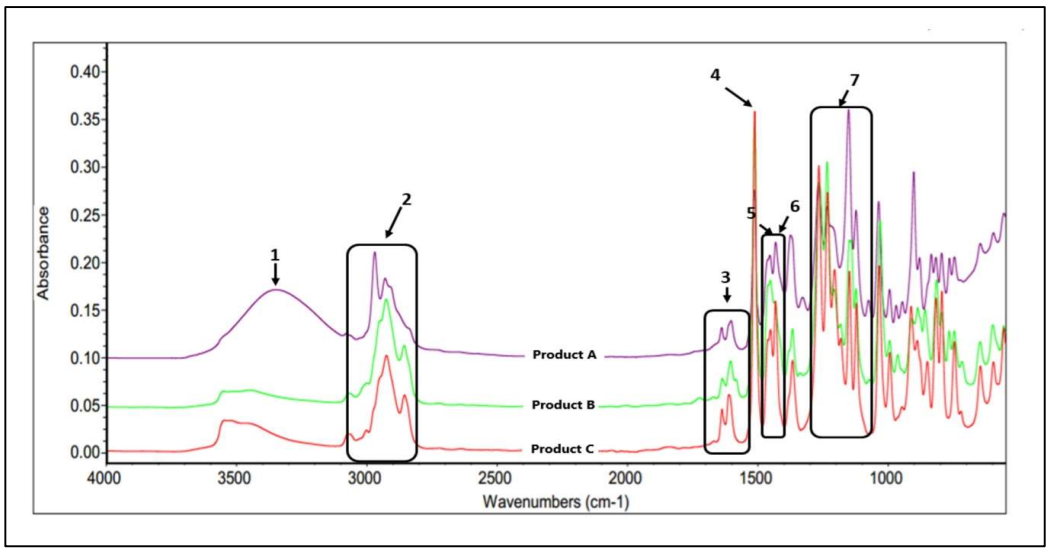

Figure 5. FTIR spectra of clove oil products in the market at wavenumbers 4000-600 cm^{-1}

Table 4. Prediction results on functional groups and vibration models of clove leaf oil products on the market

Tape	Wavenumber (cm^{-1})				Functional Groups	Vibration Models	Intensity
	Product A	Product B	Product C	Reference [17, 18]			
1	3350	-	-	3000-3600	O-H (alcohol, acid, H bonding)	hold out	Medium
2	2969	2925	2924	3000-2840	C-H	Hold out	Strong
3	1638	1636	1637	1667	C=C (para aromatic)	buckling	Weak
4	1513	1511	1511	1600 dan 1475; 1511 (eugenol acetate)	C=C (aromatic)	Hold out	Medium
5	1453	1451	1451	1469	Methylene ($-CH_2-$)	Buckling	Medium
6	1431	1433	1431	1446	Methyl ($-CH_3$)	Buckling	Medium
7	1267, 1233, 1151, 1122, and 1036	1264, 1233, 1182, 1248, 1141, and 1122	1265, 1232, 1205, 1148, 1121, and 1034	1300-1000	C-O (ether)	Hold out	Strong

Quantitative analysis with PLS

PLS regression is an analytical method that uses a linear combination of the predictor variables, not the original ones. In PLS, variables that show a high correlation with the response variable are given extra treatment because they will be more effective in the prediction process. In this method, a linear combination of predictor variables that were highly correlated with the response variable was selected to explain the existing variation. It was expected that only a few linear combinations of the predictor variables are needed to describe the majority of the variation [22].

PLS regression can be formed from a component that describes the correlation between x and y variables. Each component in the PLS regression was obtained by maximizing the covariance between the y variable and every possible linear function of the x variable. In an FTIR spectroscopy analysis, PLS is often used to extract information from complex spectra with overlapping peaks, impurities, and noise from FTIR spectroscopy instruments [35].

Before performing a PLS analysis, the first step was determining the wavenumber to be used for modelling calibration and validation by optimizing it. The wavenumber to be selected for optimization contains functional groups or fingerprint regions to determine the performance of the calibration model. It was also evaluated to provide a good correlation between the actual values of clove leaf oil, products A, B, and C oil, and turpentine oil to the predicted value using FTIR spectroscopy, expressed by the high coefficient of determination (R^2) close to 1. The R^2 value can be used to validate the accuracy parameter because R^2 describes the closeness of the predicted value to the actual value. Furthermore, the wavelength used during the analysis was selected based on the lowest error value in the calibration model, which was expressed by the Root Mean Standard Error of Calibration (RMSEC) [36, 29].

PLS connects two data matrices, x (actual value) and y (predicted value), with a linear multivariate model. The regression equation is used to find an expression that fits the data, where the linear regression equation model y=bx+a, y describes levels predicted with an FTIR spectrophotometer. The value of b is the slope of the straight line, x is the actual sample content, and a is the straight line intercept of the y-axis [37]. The optimization of wavenumber in clove oil is presented in Table 5.

Table 5. Wavenumber optimization for PLS multivariate calibration shows the relationship between the actual value (x-axis) and the predicted value (y-axis)

Wavenumber (cm^{-1})	Coefficient of Determination (R^2)	Regression Equation	RMSEC (%)
900-800	0.9991	y=0.9991x+0.0680	0.57
1300-1100	0.9908	y=0.9908x+0.7185	1.87
1400-1320	0.9955	y=0.9955x+0.3476	1.29
2900-2800	0.9993	y=0.9993x+0.0484	0.48
2960-2860	0.9998	y=0.9998x+0.0096	0.22

Multivariate calibration with the PLS method will provide relevant and specific spectral information based on the chemical properties of the calibration model at certain wavenumbers. Therefore, the selection of wavenumbers is based on optimization results which provide a linear relationship between the actual value and the predicted FTIR value [38]. As shown in Table 5, the wavenumbers used for calibration and validation of PLS modelling are 2960-2860 cm^{-1}. The relationship curve between the actual value (x-axis) and the predicted value (y-axis) of the calibration model is shown in Figure 7a.

The next process is calibration modelling at wavenumbers 2960-2860 cm^{-1}. The results, as shown in Figure 14 obtained the regression equation y=0.9998x+0.0096 and a coefficient of determination (R^2) of 0.9998. This indicated that the independent variable (x-axis) can explain 99.98% of the dependent variable (y-axis), with an RMSEC value of 0.22%. Meanwhile, the large value of the R^2 and the small RMSEC illustrate that the accuracy and precision of the developed model are acceptable.

The prediction model that has been made is evaluated using the cross-validation method by applying the leave-one-out technique. In this technique, one piece of data is deleted, and a new model is created with the remaining data. This validation is known as internal validation, where the parameter is the Root Mean Square Error of Cross Validation (RMSECV) value. Meanwhile, the validation result is said to be good when it produces a low RMSECV value, and the R^2 is close to 1. The curve of the relationship between the actual value (x-axis) and the predicted value (y-axis) using internal validation is shown in Figure 7b. Based on this curve, R^2 is 0.9992, and the RMSECV value is 0.76%, with a regression equation of y=0.9992x+1.5617. A small RMSECV value indicates that the error is getting smaller. Hence, the model built has better capabilities.

The next step is to carry out external validation to determine whether the validation model is applied to the new sample. Evaluation can be conducted by calculating the RMSEP value and the R^2. A low RMSEP value and an R^2 value close to 1 illustrate a successful creation of a regression model. The relationship curve between the actual value (x-axis) and the predicted value (y-axis) using external validation is shown in Figure 7c.

The R^2 obtained from the above curve is 0.9945, and the RMSEP value is 1.20%, with a regression equation of y=0.9945x+0.4918. The results of the external validation relationship curve, where the RMSEP value is getting smaller and the R^2 is close to 1, indicate that the validation model created can be applied to new samples.

Accuracy is expressed from R^2, where a value close to 1 illustrates that the relationship between the actual and

the predicted values is well formed, and the precision is improving. Precision was indicated by the smaller value of RMSEC, RMSECV, and RMSEP, showing that the model error was minimal. Based on several parameters, namely calibration, internal validation, and external validation, the value of $R^{2,}$ which was close to 1, showed better results. Meanwhile, smaller RMSEC, RMSECV, and RMSEP values described greater accuracy and higher precision in both models were made.

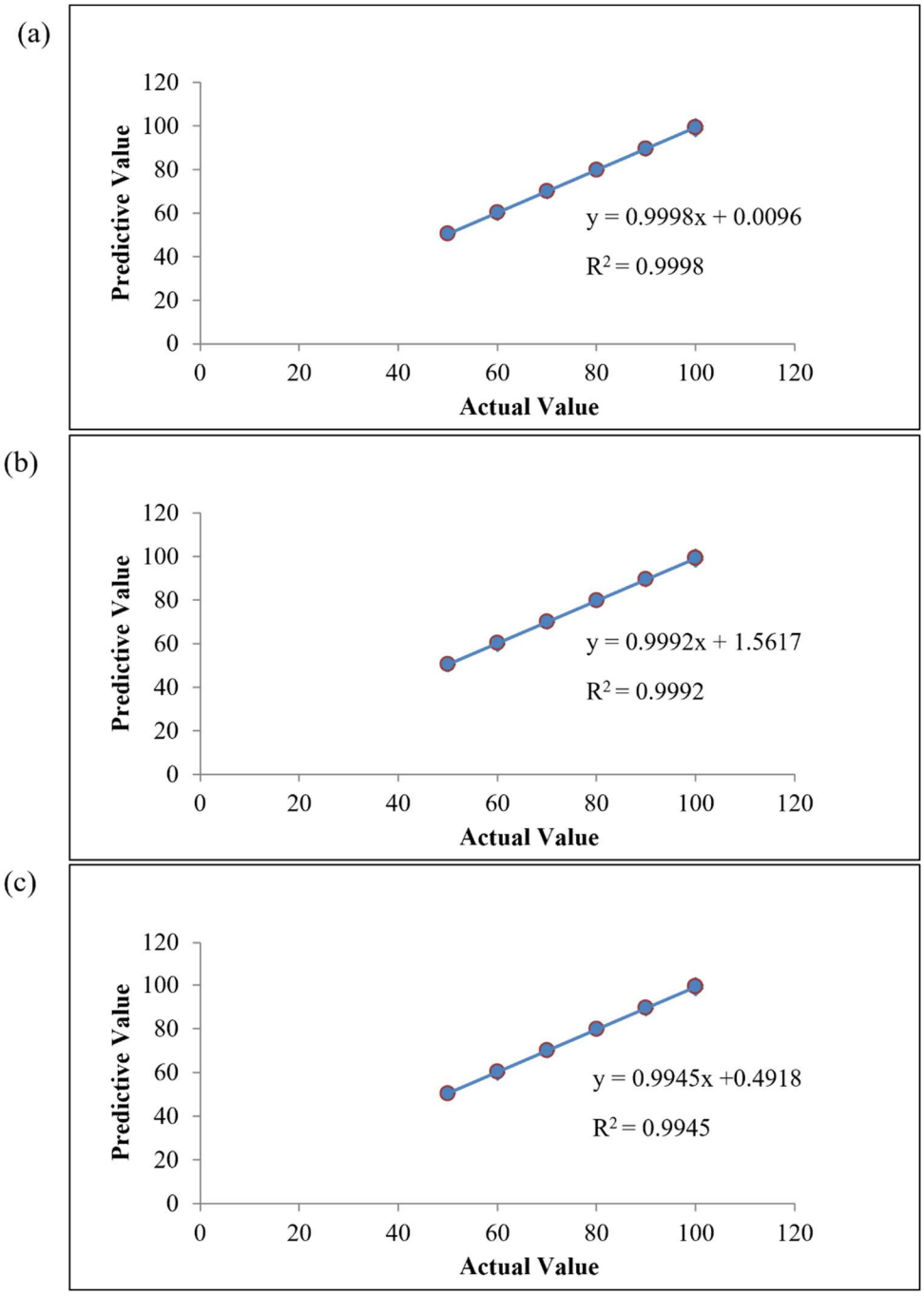

Figure 2. Relationship curves between actual values (x-axis) and predicted values (y-axis) of (a) calibration model, (b) internal validation model, and (c) external validation model at wavenumbers 2960-2860 cm^{-1}

Analysis with PCA

The FTIR spectra were analyzed using PCA, which is a data interpretation method that is performed by predicting data. This method reduces the number of variables in the matrix to generate new variables while retaining data information. The new variable is in the form of a score or main component [22]. PCA can reduce multivariate data when there is a correlation between variables. It also can be used for grouping data because samples with similar PC have similar Physicochemical properties [23].

The wavenumber that had been previously optimized was selected for PCA and used for quantitative analysis, namely 2960-2860 cm^{-1}. This wavenumber was selected for PCA modelling because of its ability to provide good separation of the samples to be evaluated, namely clove leaf oil, products A, B, and C oil, as well as turpentine oil as a forgery. The output of PCA analysis from FTIR is shown in Table 6. The results showed that analysis with PCA at wavenumbers 2960-2860 cm^{-1} reduced the initial data, namely 97 PC. This can be explained with three new variables, namely PC1, PC2, and PC3, capable of providing 100% data information. This can be seen from the eigenvalue>1, indicating that the data affect and correlate [24].

Table 6. Output PCA analysis of component profiles on the clove leaf essential oil, turpentine oil, product A, product B, and product C at wavenumbers of 2960-2860 cm^{-1}

Eigen analysis of the correction matrix											
Eigenvalue	82.235	17.151	4.552	0.054	0.008	0.001	0.000	0.000	0.000	0.000	0.000
Proportion	0.791	0.165	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cumulative	0.791	0.956	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Eigenvalue	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Proportion	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cumulative	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

A score plot comparison of clove oil and turpentine oil using PCA is shown in Figure 8a. The analysis with PCA showed that both samples are well separated, as indicated by the grouping in different quadrants, with a latent variable, namely the PCA score plot. Samples with the same PC values are similar in their physicochemical properties [39]. The grouping of pure clove leaf oil, products A, B, C, and turpentine oil was carried out. Three oil samples on the market claim to contain 100% pure clove leaf oil. The results of the PCA score plots in Figure 9b showed that 4 quadrants distinguish between clove leaf oil, turpentine oil, as well as products A, B, and C.

Based on the results of the score plot, product A oil was in the same quadrant as pure clove leaf oil. This is shown in the clove leaf oil profile analysis by GC-MS and using PCA chemometrics. These results indicated that product A had the same physical and chemical characteristics as clove leaf oil, while products B and C were in different quadrants. Based on this analysis results, PCA was successfully used to classify clove leaf oil, products A, B, and C, as well as turpentine oil. Meanwhile, future investigation regarding the main components in products A and B required further analysis such as using PCR.

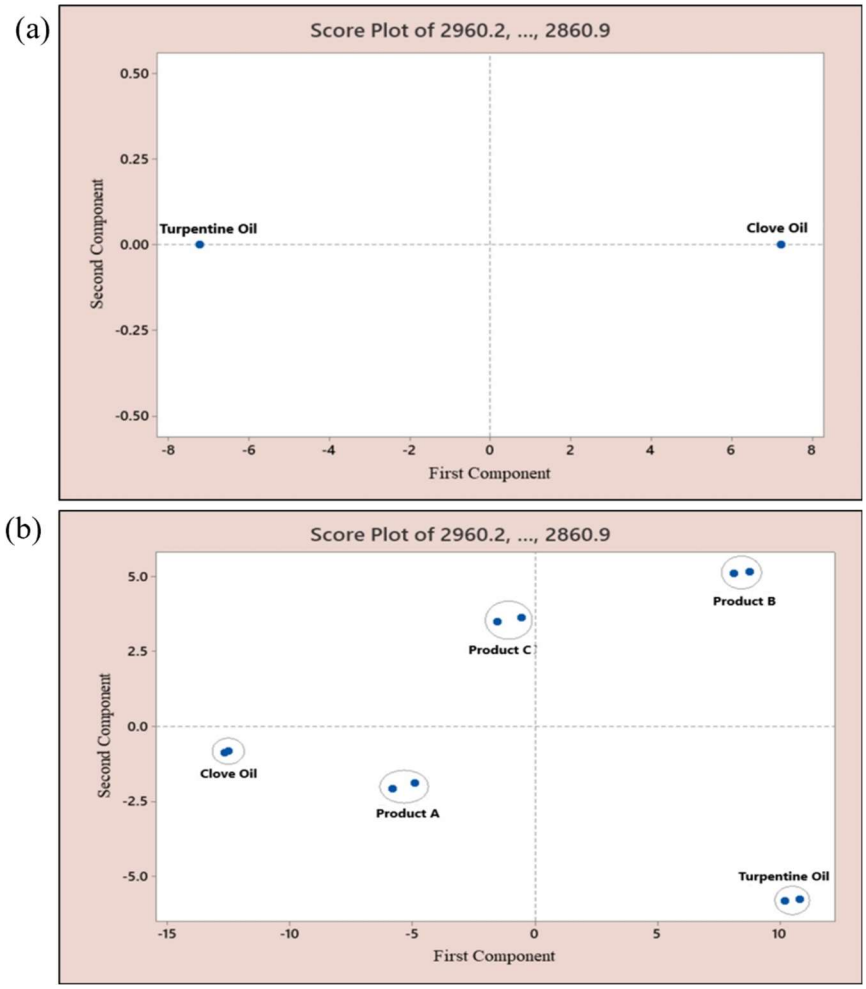

Figure 8. PCA Score Plot of FTIR (a) clove leaf oil and turpentine oil (b) clove leaf oil, turpentine oil and products A, B, C

Conclusion

The chemical components in the clove leaf oil were eugenol (3-Allyl-6-methoxyphenol), trans-Caryophyllene, alpha-Humulene, alpha-Copaene, delta-Cadinene, and Caryophyllene oxide. The results of grouping clove leaf oil using PCA based on the type of oil component showed that product A and distillate clove leaf oil were in the same quadrant. Therefore, both samples had similar physical and chemical properties. Clove leaf oil profile analysis using FTIR at wavenumbers 2960-2860 cm^{-1} with combined PLS yielded method validity results as indicated by R^2, RMSEC, RMSECV, and RMSEP values of 0.9998, 0.22%, 0.76%, and 1.20%, respectively. The results of PCA multivariate analysis showed that PCA can classify leaf oil, turpentine oil, and products A, B, and C. The authentication of oil products in the market, namely A, B, and C shows that no clove leaf oil products contain adulterated turpentine oil.

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