

Authentication of citrus peel oils from different species and commercial products using FTIR Spectroscopy combined with chemometrics

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Abstract

Citrus fruit is widely grown in Indonesia. The skin of the fruit contains essential oils which can be used as aromatic ingredients in the perfume, pharmaceutical and culinary industries. Orange peel oil, extracted from different species, has different organoleptic qualities, specific gravity, refractive index value, acid number, ester number and solubility in 96% ethanol. This research aims to determine the differences in the essential oil components of sweet orange, lime and lemon peels and to determine the composition of commercial orange peel oil products. The method used to determine the differences in orange peel oil components is ATR-FTIR spectroscopy combined with two chemometric methods, PLS and PCA, to prove the differences quantitatively and qualitatively. The results of the PLS combination FTIR analysis showed that the most optimal wave number was shown in the 1450–1650 cm^{-1} area with an RMSEC value of 0.00553, then continued with PCA which showed the separation between samples. Results confirmed that the chemometric model effectively differentiated between essential oils from sweet orange, lime and lemon peels and those available on the market.

Keywords

chemometrics, essential oil, ATR-FTIR, orange peel oil

Introduction

Citrus is a category of tropical fruits found in nearly all regions in Indonesia at an altitude of approximately 400 m above sea level (Yustinah and Fanandara 2016). The peel is part of the fruit that remains minimally utilised or repurposed. In addition to amino acids and citric acid (Mujdalipah et al. 2020), it predominantly contains essential oils, which are widely used in the perfume or fragrance, cosmetics, medicine and food industry (Cahyati et al. 2016).

Essential oils, also known as ethereal oils or volatile oils, are easily evaporated compounds that are soluble in

organic solvents, but insoluble in water and are natural extracts of plant parts, such as flowers, leaves, wood, seeds and fruit peels (Yuda Pratama et al. 2016; Kartika Fitri and Proborini 2018). Citrus peel essential oils contain limonene, linalool, α -pinene, β -pinene, myrcene, octanal and decanal. Limonene and linalool are the two main components of the essential oils of sweet orange peel. Linalool has numerous pharmacological effects, such as anti-aging, antioxidant, antiviral and antibacterial (Suardhika et al. 2018). Peels of different citrus species have dissimilar chemical makeups, resulting in essential oils with varied tangy smells (Mujdalipah et al. 2020; Fadilah et al. 2021).

Fourier transform infrared spectroscopy (FTIR) has been used to analyse the compositional differences of essential oils derived from sweet orange, lime and lemon peels. This method can determine if they have been mixed with oils of lower quality, such as turpentine oil (Ledita et al. 2019). In Indonesia, it is particularly instrumental in detecting adulterations because there is no nationally acceptable standard (i.e. SNI) controlling their commercial use and quality as deodorisers in oil products (Latifah et al. 2023). To ensure no adulterated oils circulating in the market, it has been suggested that the identification analysis combines FTIR with the chemometrics partial least square (PLS) and principal component analysis (PCA).

FTIR is a qualitative method used extensively to identify naturally occurring compounds with multiple components (Rohman and Salamah 2018) because it is simple, easy and fast. FTIR can classify functional groups from the characteristics of their spectral fingerprint regions, comprising unique wavenumbers and chemical vibrational bonds that reflect energy absorptions by functional groups upon exposure to FTIR radiation. Chemometric methods are, however, needed to interpret the complex pattern of the obtained FTIR spectrum, especially in the fingerprint region (Puspitasari et al. 2021).

Oil authentication using a combination of chemometric analysis methods has been developed to detect fraud in the sale of essential oils (Syafri et al. 2022). There are several studies examining orange peel oil, one of which is kaffir lime peel oil, the quality of which has been tested using compound analysis (Latifah et al. 2023) and detection of lemon oil using the FTIR spectroscopy method combined with chemometrics (Cebi et al. 2021). Therefore, researchers are interested in developing authentication for several species of orange peel to detect differences in the essential oil composition of sweet orange, lime and lemon peels, as well as detecting components of commercial orange peel oil products.

Materials and methods

Chemicals and materials

The research used samples of citrus fruit peels collected from sweet orange, lime and lemon plants grown in Yogyakarta, turpentine oil (Brataco) and commercial oil products denoted as A, B, and C purchased at pharmacies in Yogyakarta.

Sample preparation and distillation

Citrus peel samples were cut into 2 cm × 2 cm pieces and dried at 40–50 °C for 24 hours (Suardhika et al. 2018). The dried peels were steam-distilled and then the derived oil was purified by adding anhydrous sodium sulphate (Na₂SO₄).

Quality testing

The qualities of sweet orange, lime and lemon peel oils were characterised by their organoleptic properties (colour and odour), specific gravities, refractive index values, solubilities

in 96% ethanol, acid numbers and ester numbers. Specific gravity was determined using a 25 µl pycnometer. The refractive index was directly calculated from the oil's refractive angle measured with an Abbe refractometer. The three oils were each dissolved in 96% ethanol to determine their solubility.

To calculate the acid numbers of sweet orange, lime and lemon peel oils, 0.4 g of the oil was first mixed with 10 ml of ethanol and 5 drops of 1% phenolphthalein indicator. The mixture was titrated with 0.01 N sodium hydroxide (NaOH) until the colour turned pink (Zaimah 2016). For the ester numbers, 0.4 g of the oil was mixed with 25 ml of potassium hydroxide (KOH) solution and boiling stones and then refluxed. The yield was added with 5 drops of 1% phenolphthalein and titrated with 0.5 N hydrochloric acid (HCl) until the colour changed (Latifah et al. 2023).

Content analysis using ATR-FTIR spectroscopy combined with PLS and PCA

For analysing oil samples, these samples were prepared in a volume of 5 ml: 100% v/v distilled sweet orange peel oil (MKJM), 100% v/v distilled lime peel oil (MKJN), 100% v/v distilled lemon peel oil (MKJL), 100% v/v turpentine oil (MT) and MKJM:MT at five concentrations (90:10, 80:20, 70:30, 60:40 and 50:50 v/v) (Table 1), using an ATR-FTIR spectrophotometer (Andriansyah et al. 2022).

Table 1. Concentrations of turpentine oil (MT) and sweet orange peel oil (MKJM) blends for calibration and validation data sets.

No	MT (%)	MKJM (%)	MT:MKJM (ml)		
			MT	MKJM	MT:MKJM
1	10	90	0.5	4.5	5
2	20	80	1.0	4.0	5
3	30	70	1.5	3.5	5
4	40	60	2.0	3.0	5
5	50	50	2.5	2.5	5

PCA and PLS were used to qualitatively analyse the FTIR data using multivariate calibration in the MINITAB 19 programme. The data processed were the original absorbance spectra and the spectra consisting of partial or entire absorbance data (according to the segmentation of the spectral area) (Rohman and Salamah 2018).

Results and discussion

Quality analysis results

Chopping citrus peels can open the oil glands and facilitate evaporation and reducing their particle size increases the surface area during distillation (Yustinah and Fanandara 2016). This study distilled dried citrus peels because drying can open the pores to allow quick evaporation, resulting in greater oil yields than fresh peels (Muhtadin et al. 2013). Steam distillation was selected because it produces constant steam and heat that accelerate distillation and can prevent essential oils from degrading (Nugraheni et al. 2016). The citrus peel oil yields from the distillation are shown in Table 2.

Table 3 shows the different qualities of the distilled oils, as evident in their organoleptic characteristics, specific gravities, refractive index values, acid numbers, ester numbers and solubilities in 96% ethanol. Their varying colours and visual appearances prove that different citrus species produce oils with dissimilar organoleptic properties. Sweet orange peel oil (0.846 ± 0.003 g/ml) had similar specific gravity to kaffir lime peel oil, 0.84028 g/ml (Latifah et al. 2023).

In essential oils, acid numbers indicate quality stability. The higher the acid number, the more carboxylic acids are formed due to oxidation of the oil's aldehyde component (Latifah et al. 2023). Lemon peel oil had the highest acid number (indicating lowest stability) and ester number. Essential oils with a higher ester number have a stronger and longer scent (Latifah et al. 2023).

Solubility testing in 96% ethanol showed that sweet orange and lemon peel oils had more polar compounds than lime peel oil. The more polar compound the oil has, the more easily dissolved it will be in ethanol (Latifah et al. 2023).

Table 2. Sweet orange, lime and lemon peel oil yields.

Dried Simplicia	Weight of dried simplicia (g)	Produced oil volume (ml)	Yield (%)
Sweet orange peel	534.00	46.09	8.68
Lime peel	778.37	12.00	1.54
Lemon peel	223.17	6.50	2.91

Table 3. Quality test results of sweet orange, lime and lemon peel oils.

Test parameter	Sweet orange peel oil (Cantika et al. 2023)	Lime peel oil	Lemon peel oil
Organoleptic:			
Colour	Clear yellow	Solid yellow	Yellow
Odour	Fresh and zesty (typical citrus scent)	Zesty (typical lime scent)	Distinctively fresh lemon scent
Specific gravity (g/ml)	0.846 ± 0.003	-	-
Refractive index (25.4 °C)	1.4699 ± 0.00000	1.4725 ± 0.00010	1.4714 ± 0.00005
Acid number (mg/g)	1.10 ± 0.10	1.80 ± 0.05	4.16 ± 0.15
Ester number (mg/g)	11.20 ± 0.03	12.62 ± 0.02	16.83 ± 0.03
Solubility in 96% ethanol	1:2	1:3	1:2

Spectral and compositional profiles of the citrus peel oils

FTIR spectroscopy was conducted to qualitatively analyse the content of sweet orange, lime and lemon peel oils. FTIR spectrophotometry is a fast, simple and non-destructive analysis that identifies and displays the chemical properties of a sample in the form of spectra (Puspitasari et al. 2021). Fig. 1 shows the ATR-FTIR spectral profiles of sweet orange, lime and lemon peel oils measured in triplicate. These profiles indicated typical and similar spectral patterns, with some differences in the absorbance value.

Table 4 summarises the functional groups identified from interpreting the spectral data. Based on wavenumbers and absorbance values, it was found that sweet orange, lime and lemon peel oils are composed of similar functional groups. This was further confirmed by comparing these spectra with that of limonene in the FTIR software library. The comparison revealed that sweet orange and lemon peel oils had a hit-quality index of 958 and 921, respectively, meaning both essential oils are dominantly made up of limonene (Megawati and Kurniawan 2015). Meanwhile, the hit-quality index of the lime peel oil was only 741, indicating that limonene is present in smaller concentrations than in the previous two oils.

Adding turpentine oil to sweet orange peel oil can affect its components. Fig. 2 indicates that, when created in

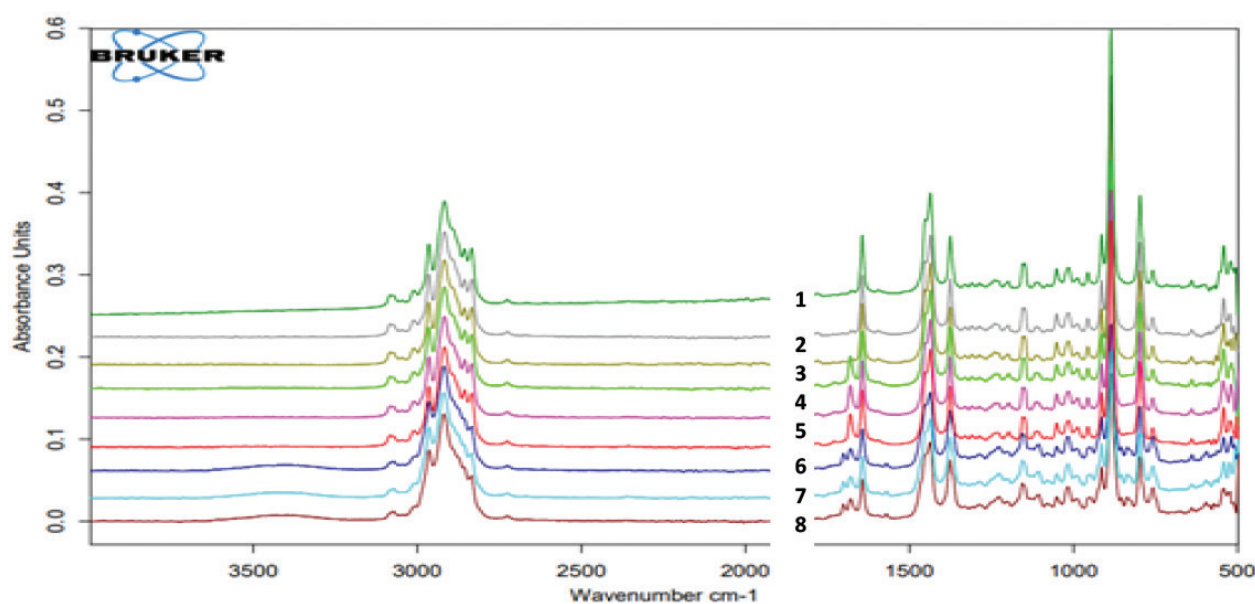
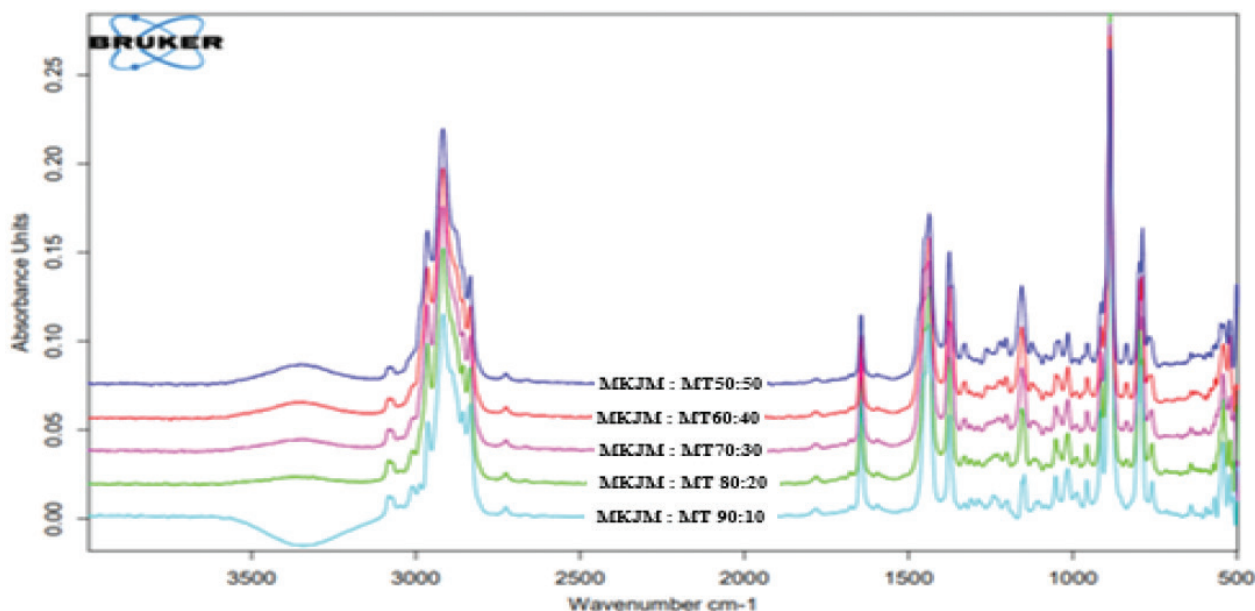


Figure 1. ATR-FTIR spectra of sweet orange (1–3), lime (4–6) and lemon (7–8).

Table 4. Functional groups of sweet orange, lime and lemon peel oils interpreted from the ATR-FTIR spectra.

No.	Wavenumber (cm ⁻¹)			Reference wavenumber (cm ⁻¹) (Winter 1984)	Intensity	Functional group
	Sweet orange peel oil (Cantika et al. 2023)	Lime peel oil	Lemon peel oil			
1	1436	1490	1457	1450–1650	Strong	C-H aromatic
2	1643	1645	1644	1640–1680	Medium	C=C
3	2918	2860	2917	2850–2975	Medium	C-H aliphatic
4	1716	1700	1716	1660–1820	Medium	C=O carbonyl

**Figure 2.** ATR-FTIR spectra of sweet orange peel oil (MKJM) mixed with turpentine oil (MT) at different concentrations.**Table 5.** Functional groups of three commercial citrus oils products interpreted from the ATR-FTIR spectra.

Product A	Wavenumber (cm ⁻¹)			Functional group	Vibration type	Intensity
	Product B	Product C	Reference			
-	3450	-	3000–3600	O-H (alcohol, acid, H bonding)	Bend	Medium
2950	-	2950	2850–2975	C-H	Stretch	Strong
1680	-	1680	1640–1680	C=C	Stretch	Weak
1490	-	1490	1450–1650	C-H aromatic	Stretch	Medium

different concentrations, this mixture produced spectra with varying intensities of absorbance. In addition, this study also analysed the ATR-FTIR spectra of three commercial citrus oil products (Fig. 3). These products mostly showed similar spectral profiles to sweet orange, lime and lemon peel oils, with a few differences in the absorbance values indicative of O-H and C-H groups, which were not found in the distilled oils. The interpretation results of the commercial products' spectra are summarised in Table 5.

Table 6. Wavenumber optimisation for PLS multivariate calibration by correlating the actual values (x -axis) and the predicted values (y -axis).

Wavenumber (cm ⁻¹)	Coefficient of determination (R ²)	Regression equation	RMSEC
900–700	0.99995	$y = 0.9999x + 0.0118$	0.24959
1200–1000	0.99999	$y = 0.9999x + 0.0003$	0.08989
1650–1450	1	$y = 1x + 0.00006$	0.00553
1750–1651	0.99933	$y = 0.9993x + 0.0425$	0.93605
3000–2900	0.99992	$y = 0.9999x + 0.0029$	0.3118

FTIR spectroscopy is often combined with PLS to extract information from complex spectral data that consists of overlapping peaks, impurities and noise from the spectroscopy instrument (Rohman et al. 2021). The combined process will provide spectral information relevant to certain properties of the observed variables. This study used PLS modelling to quantify total limonene using five spectral regions: 900–700, 1200–1000, 1650–1450, 1750–1651, and 3000–2900 cm⁻¹. PLS regression model was developed to find a linear correlation between the absorbance value of each MKJM:MT concentration as the x variable (predictor) and the concentration of MKJM as the y variable (response).

The model's accuracy was decided from the coefficient of determination and the error value (Rasyida et al. 2014). Accordingly, the wavenumbers were optimised to improve the model's prediction accuracy. Table 6 shows R² = 1 for the wavenumbers 1650–1450 cm⁻¹, meaning the model provides the best prediction in this region where the predicted and actual values are identical. In addition, its root mean standard error of calibration (RMSEC) was

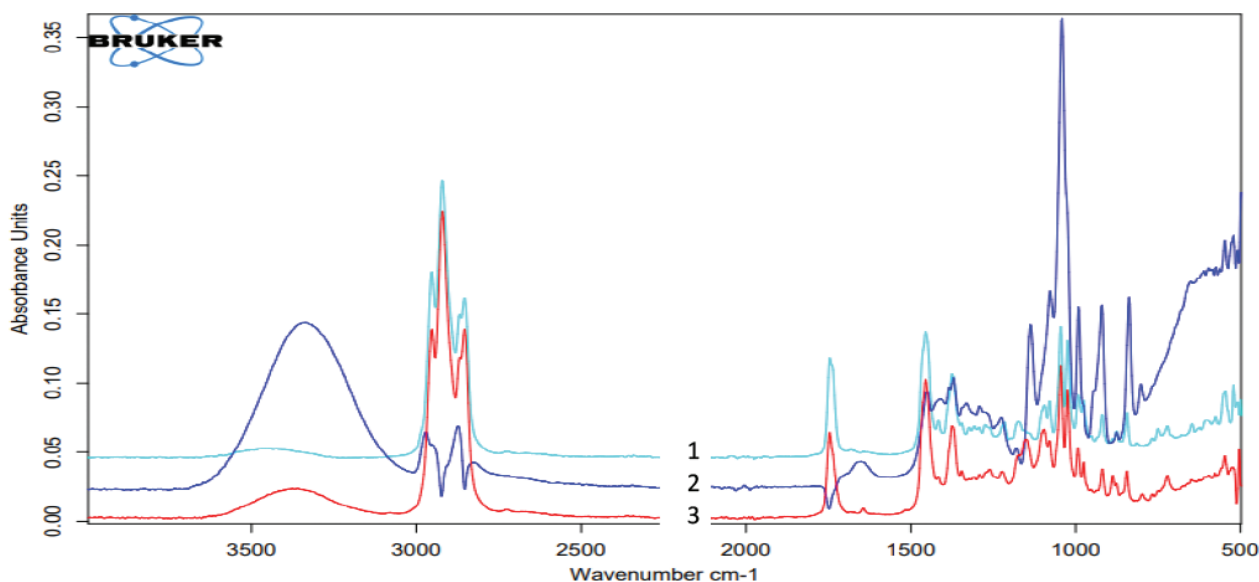


Figure 3. ATR-FTIR spectra of three commercial oil products in the 3500–500 cm^{-1} region. (1: Product A, 2: Product B, 3: Product C).

the smallest. Smaller RMSEC means the model has a higher accuracy (Rasyida et al. 2014). With a high R^2 and low RMSEC, the model developed to authenticate sweet orange, lime and lemon peel oils is deemed accurate and precise (Putri et al. 2021).

Afterwards, the prediction model was evaluated by cross-validation using the leave-one-out technique, i.e. removing one piece of data and creating a new model with the remaining data. This method, also known as internal validation, produces root mean square error of cross-validation (RMSECV). The prediction model is acceptable if the internal validation process yielded low RMSECV and a coefficient of determination (R^2) close to 1. Based on the correlation curves shown in Fig. 4, the model had $R^2 = 0.9993$ and RMSECV = 0.8104, with a regression equation of $y = 0.9915x + 1.5556$. The small RMSECV value indicates that it produces a good prediction with low error.

Then, an external validation was conducted to determine whether the prediction model could be applied to new samples, based on its R^2 value and root mean square error of prediction (RMSEP). Fig. 4 shows that the external validation produced a regression equation of $y = 0.9989x + 0.0428$, with $R^2 = 0.9981$ and RMSEP = 1.1777. The RMSEP and R^2 (close to 1) indicated that the prediction model can be applied to new samples. From the

calibration, internal validation and external validation results, it can be inferred that the developed model has acceptable results, where the predicted and actual values are nearly identical (R^2 close to 1), as well as good accuracy and precision (small RMSEC, RMSECV and RMSEP).

Following the PLS modelling was principal component analysis (PCA), a sample grouping method (Putri et al. 2021). PCA determines principal components, which are a linear combination of the actual variables. PCA can also facilitate the visualisation of data grouping and initial evaluation of similarities between groups and determine the factors behind an observed pattern through correlations between chemical or physical properties (Dian et al. 2015). The PCA results in the 1650–1450 cm^{-1} region reduced the dimension of the initial data from 100 PCs to four new variables (PC1, PC2, PC3 and PC4) without losing information. The ability of a PC to explain variance in the data is expressed as eigenvalues, which can reveal correlation or covariance between variables (Rohman et al. 2021).

The PCA score plot in Fig. 5 shows lemon and lime peel oils in the same quadrant. Additionally, Products A and C were in the same quadrant as turpentine oil, meaning they possess similar physical and chemical characteristics. In contrast, Product B was found in a different quadrant. This analysis shows that PCA can

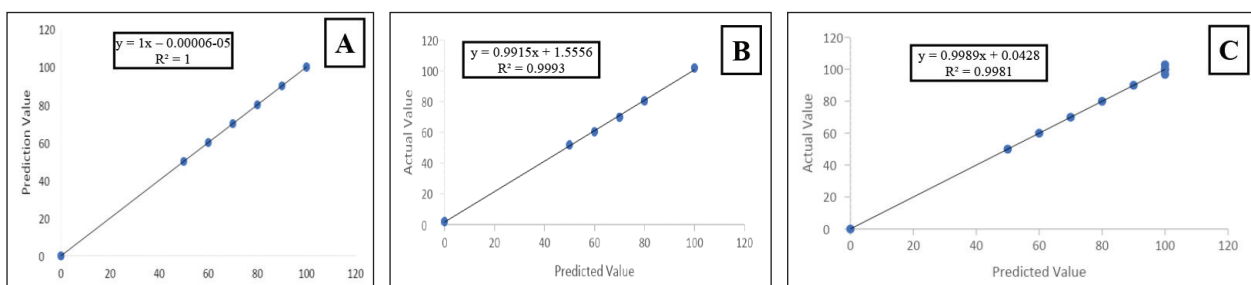


Figure 4. Correlation curves between the actual values (x -axis) and the predicted values (y -axis): (A) the model's calibration using the 1650–1450 cm^{-1} region (optimised wavenumbers), (B) internal validation and (C) external validation.

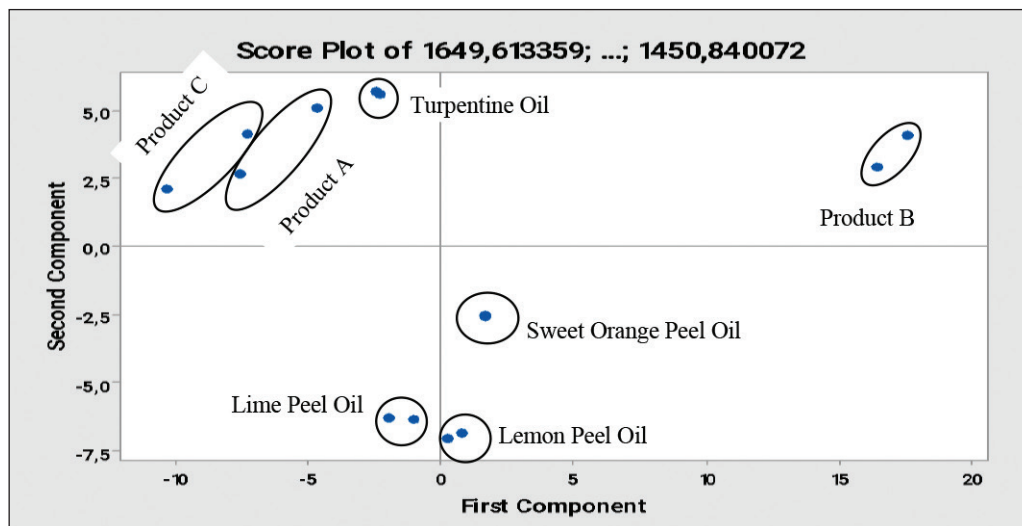


Figure 5. PCA score plot of the sweet orange, lime, lemon peel oils, turpentine oil and commercial oil products A, B and C.

distinguish between sweet orange, lime, lemon peel oils, turpentine oil and commercial oil products. Adjacent points in the score plot indicate physicochemical similarity based on the main components of the samples (Widyastuti et al. 2020).

Conclusions

Sweet orange, lime and lemon peel oils have different organoleptic qualities, specific gravities, refractive index values, acid numbers, ester numbers and solubilities. ATR-FTIR spectroscopy combined with chemometrics

(PLS and PCA) can effectively distinguish between citrus peel oils extracted from different varieties and determine whether they share similar compositions with commercial citrus oil products.

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