FTIR SPECTROSCOPIC CHARACTERIZATION OF VIRGIN COCONUT OIL PRODUCED BY FERMENTATION USING *Moringa oleifera* LEAVES AND BAKER’S YEAST

Grace Loupatty[1], **Ana Yeti Huwae**[2], **Diana Julaidy Patty**[1*]
[1]*Program Studi Fisika, Fakultas Sains dan Teknologi, Universitas Pattimura, Jl. Ir. M. Putuhena, Ambon, 97233, Indonesia*
[2]*Laboratorium Fisika Zat Padat Universitas Pattimura Jl. Ir. M. Putuhena, Ambon, 97233, Indonesia.*
* e-mail: dianapatty.dp@gmail.com

ABSTRACT

Virgin coconut oil (VCO) is widely recognized for its nutritional and functional properties, particularly due to the presence of bioactive compounds such as phenolic compounds that contribute to antioxidant activity. This study aims to investigate the effect of different fermentation agents, namely Moringa oleifera leaves and baker’s yeast, on the spectral characteristics and potential phenolic-related functional groups in VCO using Fourier Transform Infrared (FTIR) spectroscopy. VCO samples were produced through a fermentation extraction process using moringa leaves (VCO-K) and yeast (VCO-F). FTIR spectra in the range of 4000–400 cm⁻¹ were analyzed to identify functional groups associated with phenolic compounds and lipid structures. Both samples showed a broad O–H stretching band around 3471 cm⁻¹ (VCO-K) and 3464 cm⁻¹ (VCO-F), indicating the possible presence of hydroxyl groups related to phenolic compounds and hydrogen bonding interactions. Additional characteristic absorption bands corresponding to lipid functional groups, including C–H stretching and ester carbonyl (C=O) vibrations, were also observed. The refractive index values of VCO-K (1.454) and VCO-F (1.458) were close to reported VCO standards, indicating acceptable physicochemical quality. These findings demonstrate that FTIR spectroscopy is a useful approach for identifying molecular functional groups and investigating potential phenolic-related interactions in VCO produced using different fermentation treatments.

Keywords: *VCO, Yeast, Moringa, FTIR Spectroscopy, phenol*

INTRODUCTION

Virgin coconut oil (VCO) is a natural oil obtained from fresh coconut meat through mechanical or fermentation processes without high-temperature treatment. Chemically, VCO is composed mainly of triglycerides dominated by saturated medium-chain fatty acids (MCFAs), particularly lauric acid, along with minor bioactive components such as phenolic compounds, tocopherols, and other phytochemicals that contribute to antioxidant activity (Ng et al., 2021; Rohman & Che Man, 2009). The molecular composition and functional groups present in VCO can be effectively investigated using vibrational spectroscopy techniques, especially Fourier Transform Infrared (FTIR) spectroscopy.

FTIR spectroscopy is widely used for rapid and non-destructive characterization of organic compounds through the identification of vibrational modes associated with specific functional groups. In lipid-based materials such as vegetable oils, FTIR spectra provide information about characteristic bands corresponding to C–H stretching vibrations of aliphatic chains, carbonyl (C=O) groups of triglycerides, and hydroxyl (O–H) groups related to phenolic compounds or hydrogen bonding interactions (Amit et al., 2023; Neves & Poppi, 2020). Previous FTIR studies on VCO have mainly focused on the authentication of coconut oil, detection of adulterants, and identification of lipid functional groups in edible oils (Man & Rohman, 2013; Rohman & Che Man, 2009). However, limited studies have explored how different fermentation agents used during the extraction process may influence the vibrational spectral characteristics of VCO.

Fermentation is one of the commonly applied methods for producing VCO because it facilitates the separation of oil from coconut milk through enzymatic and biochemical processes. During fermentation, microorganisms or plant-derived enzymes can promote the hydrolysis of proteins surrounding fat globules in coconut cream, allowing the oil phase to separate more efficiently (Raudya et al., 2023). Variations in fermentation agents may influence the physicochemical properties of the resulting VCO and potentially affect the molecular interactions observable in infrared spectra.

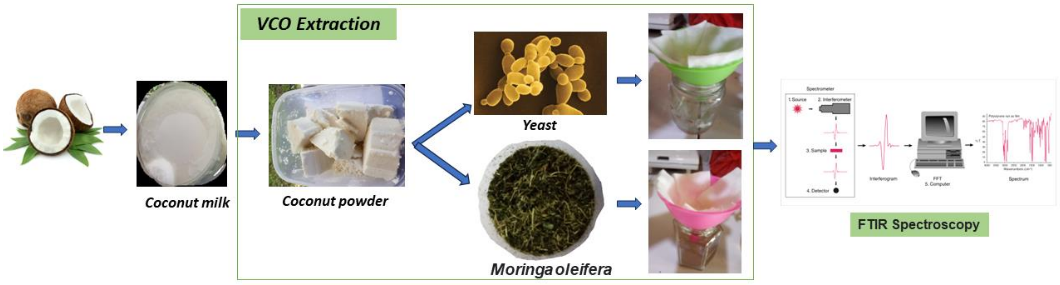

Figure 1. Scheme of extraction of virgin coconut oil (VCO) using moringa leaves and yeast with FTIR characterization

In addition to microbial fermentation, plant materials rich in phenolic compounds may contribute additional bioactive components during the extraction process. One such plant is *Moringa oleifera*, whose leaves are known to contain significant amounts of phenolic compounds with antioxidant properties (Hassan et al., 2021; Starzyńska-Janiszewska et al., 2022). Previous studies have identified phenolic-related functional groups in moringa leaf extracts using FTIR spectroscopy, particularly in the O–H stretching region associated with hydroxyl groups of phenolic compounds (Cruz-Espinoza et al., 2012; Khalid et al., 2023). However, the influence of moringa leaves used during the fermentation extraction process on the FTIR spectral characteristics of VCO has not been widely investigated.

Compared with previous studies, this research provides several contributions. First, while conventional fermentation methods typically employ microbial starters such as yeast, this study compares fermentation using baker's yeast with fermentation assisted by moringa leaves as a plant-based additive. Second, previous FTIR investigations on VCO have primarily focused on oil authentication and adulteration detection, whereas this study examines the spectral features associated with functional groups potentially related to phenolic compounds. Third, although moringa leaves have been extensively studied as sources of natural antioxidants, their potential influence on the molecular vibrational characteristics of VCO during fermentation extraction has received limited attention.

Therefore, the objective of this study is to investigate the FTIR spectral characteristics of virgin coconut oil obtained through fermentation processes using moringa leaves (*VCO-K*) and baker's yeast (*VCO-F*) (Figure 1). The analysis focuses on identifying functional groups associated with lipid structures and potential phenolic compounds, as well as examining possible molecular interactions reflected in the infrared spectra.

METHOD

2.1 Materials

The materials used in this study included coconut milk extracted from fresh mature coconuts (three medium-sized coconuts), baker's yeast (Fermipan, 11 g), fresh *Moringa oleifera* leaves (500 g), and distilled water (1–2 L). All materials were obtained from local sources in Ambon, Indonesia..

2.2 Preparation of Moringa Leaf Extract

Fresh *Moringa oleifera* leaves were obtained from the Mardika traditional market in Ambon and washed thoroughly with distilled water to remove impurities. The leaves were separated from the stems and dried in an oven at 60 °C for 30 minutes. After drying, the leaves were ground using a laboratory grinder to obtain moringa leaf powder, which was used as a fermentation additive in the VCO extraction process.

2.3 VCO Fermentation Process

Virgin coconut oil (VCO) was produced through a fermentation process using two different fermentation agents: moringa leaves and baker's yeast.

First, coconut milk was extracted from freshly grated mature coconut meat. The coconut milk was placed in a closed container and allowed to stand at room temperature for approximately 4 hours until separation between the cream and aqueous phase occurred. The coconut cream layer was then separated and stored in a refrigerator overnight until frozen.

The frozen coconut cream was subsequently removed and crushed into a fine powder to facilitate the fermentation process. The powdered coconut cream was divided into two treatment groups:

- VCO-K: fermentation using moringa leaf powder
- VCO-F: fermentation using baker's yeast

Each mixture was incubated at approximately 40 °C for 24 hours to allow fermentation to occur. After fermentation, three layers were formed: VCO oil at the top, coconut curd in the middle, and water at the bottom. The oil layer was carefully separated using a pipette and filtered through gauze and cotton to obtain clear VCO oil.

All extraction experiments were performed in triplicate (n = 3) to ensure reproducibility.

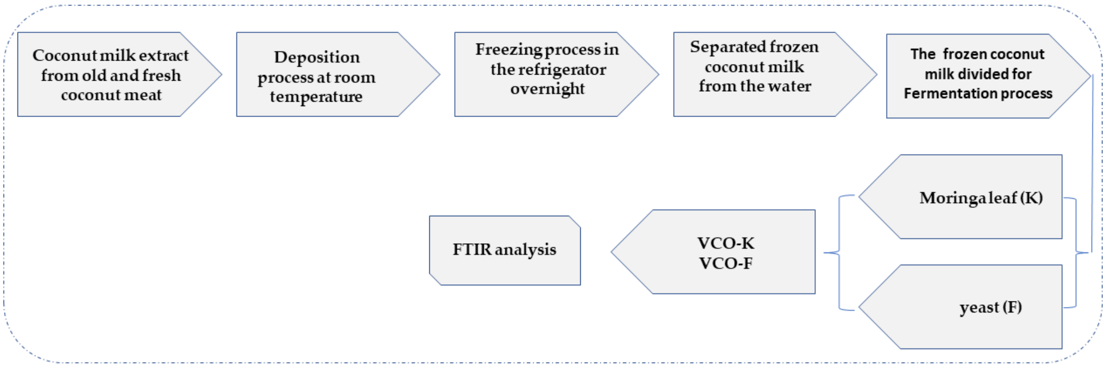

Figure 2. The fermentation process of VCO

2.4 FTIR Spectroscopic Analysis

The functional groups present in the VCO samples were analyzed using a Shimadzu 8201PC Fourier Transform Infrared (FTIR) spectrophotometer at the Chemistry Laboratory of Universitas Gadjah Mada.

Spectral measurements were carried out using the Attenuated Total Reflectance (ATR) technique. The spectra were recorded in the wavenumber range of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1} and 32 scans per sample to improve the signal-to-noise ratio.

Prior to each measurement, a background spectrum was recorded and automatically subtracted from the sample spectrum. The obtained FTIR spectra were processed using standard spectral treatment procedures, including baseline correction and normalization, to enable accurate comparison of the absorption bands among the samples.

The analysis focused on identifying characteristic vibrational bands corresponding to functional groups commonly found in lipids and phenolic-related compounds, such as O–H stretching vibrations, C–H stretching of aliphatic chains, and ester carbonyl (C=O) vibrations.

2.5 Refractive Index Measurement

The refractive index of the VCO samples was measured using an Abbe refractometer (Ivymen System) at the Chemistry Laboratory of Universitas Gadjah Mada. Measurements were performed at 23 °C.

Each sample was measured three times (n = 3), and the reported values represent the average refractive index obtained from the repeated measurements. The results were then compared with reported reference values for virgin coconut oil.

Figure 4. The VCO extraction by a fermentation process using moringa leaf (VCO-K) and yeast (VCO-F)

RESULTS AND DISCUSSION

The FTIR spectra of virgin coconut oil extracted through fermentation using moringa leaves (VCO-K) and baker's yeast (VCO-F) are presented in Figure 3. The spectra exhibit several characteristic absorption bands commonly observed in vegetable oils, corresponding to vibrational modes of lipid functional groups.

Table 1 summarizes the main FTIR absorption bands and refractive index values of VCO samples produced through fermentation using *Moringa oleifera* leaves (VCO-K) and baker's yeast (VCO-F). Both samples show a broad absorption band in the region of 3200–3600 cm^{-1}, which corresponds to the O–H stretching vibration. The peak maximum appears at approximately 3471 cm^{-1} for VCO-K and 3464 cm^{-1} for VCO-F. The presence of this broad band may indicate hydroxyl groups associated with phenolic compounds as well as hydrogen-bonded structures within the oil matrix. Similar O–H stretching bands have been reported in FTIR studies of phenolic-containing plant extracts and vegetable oils (Oliveira et al., 2016; Cruz-Espinoza et al., 2012).

The slightly stronger O–H absorption observed in the VCO-K sample may be related to the presence of bioactive compounds derived from *Moringa oleifera* leaves, which are known to contain phenolic compounds with hydroxyl functional groups (Hassan et al., 2021). However, because FTIR spectroscopy detects functional groups rather than specific molecules, the presence of phenolic compounds is inferred qualitatively rather than quantified.

The FTIR spectra also show characteristic lipid absorption bands. Strong peaks observed around 2925 cm^{-1} and 2854 cm^{-1} correspond to asymmetric and symmetric C–H stretching vibrations of aliphatic $–CH_2$ groups, which are typical of long hydrocarbon chains in triglycerides.

Another prominent band appears at 1743 cm^{-1}, which corresponds to the C=O stretching vibration of ester groups present in triglycerides. This band is a typical spectral signature of vegetable oils and indicates the presence of ester linkages in fatty acid molecules (Rohman & Che Man, 2009).

In the lower wavenumber region, absorption bands near 1465 cm^{-1} and 1377 cm^{-1} correspond to bending vibrations of CH_2 and

CH_3 groups, while bands around 1117 cm^{-1} are associated with C–O stretching vibrations in ester structures.

Infrared absorption occurs when molecular vibrations produce a change in the dipole moment of a chemical bond. In triglyceride molecules, vibrations such as C–H stretching, C=O stretching, and CH_2 bending generate characteristic absorption bands in the FTIR spectrum.

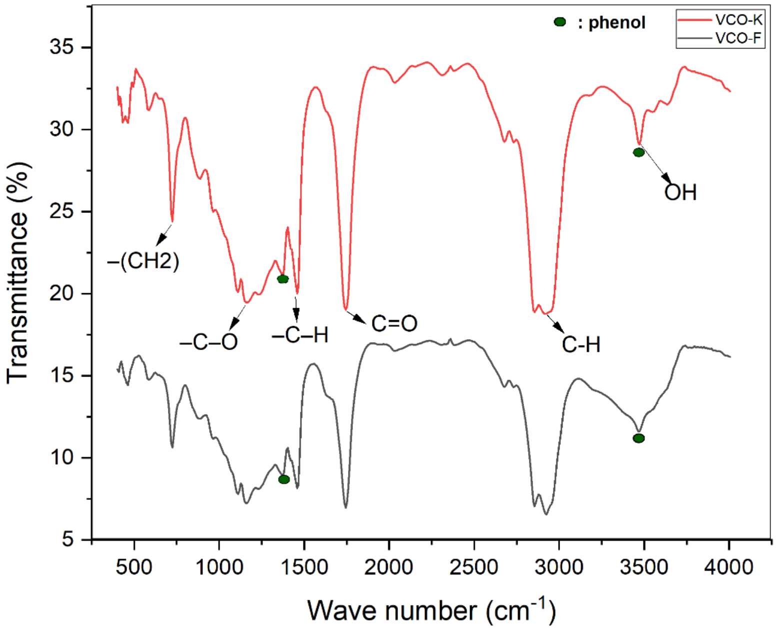

Figure 3. FTIR spectra of VCO oil extract by Moringa leave (VCO-K) and yeast (VCO-F)

Table 1. Functional Groups and Refractive index VCO oil

Sample	Wavenumber (cm^{-1})	Vibrational Mode	Functional Group	Molecular Assignment	Refractive Index (23°C)
VCO-K (Moringa fermentation)	~3471	O–H stretching (broad)	Hydroxyl group	Hydrogen-bonded O–H vibration possibly associated with phenolic compounds or residual moisture	1.454 ± 0.002
	~2925	Asymmetric C–H stretching	$-CH_2$	Long hydrocarbon chains in triglycerides	
	~2854	Symmetric C–H stretching	$-CH_2$	Aliphatic fatty acid chains	
	~1743	C=O stretching	Ester carbonyl	Triglyceride ester linkage	
	~1465	CH_2 bending	Alkane group	Methylene bending vibration	
	~1377	CH_3 bending	Methyl group	Terminal methyl groups of fatty acids	
	~1117	C–O stretching	Ester group	C–O vibration of triglycerides	
VCO-F (Yeast fermentation)	~3464	O–H stretching (broad)	Hydroxyl group	Hydrogen-bonded O–H vibration	1.458 ± 0.002
	~2924	Asymmetric C–H stretching	$-CH_2$	Hydrocarbon chains in triglycerides	
	~2853	Symmetric C–H stretching	$-CH_2$	Aliphatic fatty acid chains	

Sample	Wavenumber (cm^{-1})	Vibrational Mode	Functional Group	Molecular Assignment	Refractive Index (23°C)
	~1743	C=O stretching	Ester carbonyl	Triglyceride ester bond	
	~1465	CH_2 bending	Alkane group	Methylene bending vibration	
	~1377	CH_3 bending	Methyl group	Methyl group vibration	
	~1117	C–O stretching	Ester group	C–O vibration in triglycerides	

The broad O–H band observed in both samples can be attributed to hydrogen-bonded hydroxyl groups. Hydrogen bonding reduces the effective force constant of the O–H bond, leading to a red shift and spectral broadening in the stretching vibration region. The difference in the O–H peak intensity between VCO-K and VCO-F may therefore reflect differences in hydrogen bonding interactions or hydroxyl-containing compounds present in the samples.

Interestingly, the FTIR spectra show no significant peaks near 3008 cm^{-1} or 1654 cm^{-1}, which are typically associated with C=C stretching vibrations of unsaturated fatty acids. The absence of these peaks is consistent with the chemical composition of VCO, which contains a high proportion of saturated medium-chain fatty acids such as lauric acid (Ng et al., 2021).

Differences between the spectra of VCO-K and VCO-F may be influenced by the fermentation agents used during the extraction process. Moringa leaves contain various phytochemicals, including phenolic compounds and flavonoids, which may introduce additional hydroxyl groups into the oil system.

In addition, the freezing treatment applied during sample preparation may alter the molecular interactions within coconut cream by disrupting protein-stabilized emulsions. This process may facilitate the separation of triglyceride molecules and influence hydrogen bonding interactions detectable in the FTIR spectra.

The refractive index values of the VCO samples were measured at 23 °C. The average refractive index values were 1.454 for VCO-K and 1.458 for VCO-F, which are consistent with reported values for virgin coconut oil in the literature (Seneviratne & Jayathilaka, 2016). These values indicate that the extracted oils fall within the typical refractive index range for VCO. Although FTIR spectroscopy is useful for identifying functional groups in complex organic materials, it has limitations for quantifying phenolic compounds directly. Accurate quantification of phenolic concentration requires calibration using reference standards or complementary analytical techniques such as HPLC or spectrophotometric assays. Therefore, the present FTIR analysis provides qualitative information about hydroxyl-related functional groups rather than absolute phenolic concentrations.

CONCLUSION

This study investigated the molecular characteristics of virgin coconut oil (VCO) produced through fermentation using *Moringa oleifera* leaves (VCO-K) and baker's yeast (VCO-F) using Fourier Transform Infrared (FTIR) spectroscopy and refractive index analysis. The FTIR spectra of both samples showed characteristic absorption bands associated with triglyceride structures, including O–H stretching, aliphatic C–H stretching, ester carbonyl (C=O) stretching, and C–O vibrations typical of vegetable oils. A broad O–H absorption band observed around 3460–3470 cm^{-1} indicates hydrogen-bonded hydroxyl groups within the oil matrix. The slightly stronger O–H absorption in the VCO-K sample suggests differences in hydrogen-bond interactions that may arise from the fermentation treatment using *Moringa oleifera* leaves.

The refractive index values measured at 23 °C fall within the typical range reported for virgin coconut oil, confirming that both fermentation methods produced oil with physicochemical characteristics consistent with VCO standards. Overall, FTIR spectroscopy provides useful qualitative insight into the molecular structure and vibrational signatures of VCO obtained through different fermentation treatments. However, because FTIR analysis alone cannot quantify phenolic compounds without calibration, future studies are recommended to combine FTIR spectroscopy with chemometric modeling or complementary analytical techniques for more detailed quantitative analysis.

ACKNOWLEDGMENT

The authors would like to thank you for financially supporting this research with the PNBP Grant of the Faculty of Mathematics and Natural Sciences, Pattimura University.

Conflict of Interest. The authors declare no conflicts of interest.

REFERENCES

Aziz A A, Lim J, Ping B T Y and Ong H C 2017 FTIR spectroscopy and chemometric analysis of virgin coconut oil *Food Analytical Methods* **10** 1032–1039 https://doi.org/10.1007/s12161-016-0650-2

Coates J 2006 Interpretation of infrared spectra: a practical approach *Encyclopedia of Analytical Chemistry* **12** 10815–10837 https://doi.org/10.1002/9780470027318.a5606

Cruz-Espinoza L P, Ruiz-Cabrera M A and Ramírez-Guzmán M G 2012 FTIR characterization of phenolic compounds in plant extracts *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **97** 537–541

Guillén M D and Cabo N 1997 Infrared spectroscopy in the study of edible oils and fats *Journal of the Science of Food and Agriculture* **75** 1–11 https://doi.org/10.1002/(SICI)1097-0010(199709)75:1

Hassan F A, Mahrose K M and Attia Y A 2021 Nutritional and functional properties of *Moringa oleifera* leaves: a review *Journal of Applied Phycology* **33** 257–270 https://doi.org/10.1007/s10811-020-02239-7

Ng S P, Lai O M, Abas F, Lim H K and Tan C P 2021 Virgin coconut oil: a review on extraction methods, physicochemical properties and health benefits *Food Reviews International* **37** 111–132 https://doi.org/10.1080/87559129.2019.1603519

Oliveira R N, Mancini M C, Oliveira F C S, Passos T M, Quilty B, Thiré R M S M and McGuinness G B 2016 FTIR analysis and quantification of phenols and flavonoids of five commercially available plants used in wound healing *Materials* **9** 351 https://doi.org/10.3390/ma9050351

Rohman A and Che Man Y B 2009 Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil *Food Research International* **43** 886–892 https://doi.org/10.1016/j.foodres.2009.12.006

Rohman A, Che Man Y B and Ismail A 2014 FTIR spectroscopy combined with chemometrics for analysis of fats and oils *Food Chemistry* **158** 144–150 https://doi.org/10.1016/j.foodchem.2014.02.128

Seneviratne K N and Jayathilaka N 2016 Coconut oil: chemistry and nutrition *Asian Journal of Food and Agro-Industry* **9** 56–64 van de Voort F R, Ismail A A and Sedman J 2008 FTIR spectroscopy for the authentication of edible oils *Food Research International* **41** 815–823 https://doi.org/10.1016/j.foodres.2008.05.001

Yang H, Irudayaraj J and Paradkar M M 2005 Discriminant analysis of edible oils using FTIR spectroscopy *Food Chemistry* **93** 25–32 https://doi.org/10.1016/j.foodchem.2004.08.039