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ANALYSIS OF MEDIUM-CHAIN FATTY ACID COMPONENTS IN VIRGIN COCONUT OIL WITH THE ADDITION OF NUTMEG FRUIT FLESH EXTRACT AS IMMUNOMODULATORY BIOACTIVE COMPOUNDS

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Abstract

This study aims to produce Virgin Coconut Oil (VCO) enriched with bioactive compounds by adding nutmeg flesh extract. The research analyzes the fatty acid composition of VCO-caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid-using Gas Liquid Chromatography (GLC) while exploring its potential as an immunomodulator through literature review. A laboratory experiment was conducted by analyzing the fatty acid components of VCO with nutmeg flesh extract (Nutmeg VCO) using GLC. The results show that the Nutmeg VCO meets the quality parameters of SNI 7381:2008, with a moisture content of 0.13%, an average free fatty acid (FFA) level of 0.09%, a clear appearance, and a distinct aroma of both coconut and nutmeg. The identified fatty acid composition includes caproic acid (0.46%), caprylic acid (7.70%), capric acid (6.24%), lauric acid (50.59%), myristic acid (18.52%), palmitic acid (7.85%), stearic acid (2.26%), and linoleic acid (1.47%). Nutmeg VCO contains bioactive compounds such as phenols, tocopherols, and flavonoids, which have potential immunomodulatory effects. Additionally, due to its fermentation-based production without microbial additives, VCO naturally contains lactic acid bacteria. These bacteria produce bacteriocins that exhibit antimicrobial, antiviral, and antifungal properties, potentially inhibiting pathogenic microorganisms, including COVID-19. This fermentation method represents a novel approach in VCO production.

Key words: Virgin coconut oil, Fatty acids, Nutmeg fruit flesh, Immunomodulator, Bioactive compounds, Nutmeg extract

Introduction

Virgin Coconut Oil (VCO) is derived from the flesh of mature coconuts and has numerous benefits, making it an important product that can be processed into oil (Koleangan, 2021). VCO is widely recognized as a natural product with various health benefits. The medium-chain fatty acids (MCFA) in VCO, such as lauric acid, possess antiviral, antibacterial, and antifungal properties. These characteristics make VCO a potential immunomodulator, a substance that can modify the body's immune response.

Recent studies on VCO indicate that bioactive compounds in nutmeg flesh, such as myristicin, elemicin, and eugenol, have the potential to enhance immunomodulatory activity. These compounds are known for their anti-inflammatory and antioxidant properties, which can support the immune system.

The combination of VCO with nutmeg flesh extract is expected to enhance VCO's effectiveness as an immunomodulator. This study aims to analyze the fatty acid composition of VCO, including both medium- and long-chain fatty acids, in VCO enriched with nutmeg flesh extract and to assess its immunomodulatory potential.
The research questions addressed in this study are:

(a) What is the fatty acid composition of VCO with the addition of nutmeg flesh extract?
(b) What is the immunomodulatory potential of VCO enriched with nutmeg flesh extract?

This study is expected to contribute to the advancement of knowledge, particularly in the fields of food technology and health, by exploring the use of VCO and nutmeg flesh extract as bioactive compounds with immunomodulatory potential.

Material and Methods

The materials used in this study include nutmeg flesh at full maturity and mature coconuts (*Cocos nucifera*, 11 months old). VCO was produced using a modified wet fermentation method by first extracting coconut milk with the addition of clean water and coconut water in a 1:1 ratio.

The equipment used includes a thermometer, round-bottom flask, test tubes, electric oven, water bath, evaporator, reflux apparatus set, spectrophotometer (517 nm), gas-liquid chromatography (Shimitzu, Japan), coconut peeler (Shark Paper: Honda, Japan), coconut meat grater (Shark Paper: Honda, Japan), and coconut meat press (Shark Paper: Honda, Japan).

Preparation of nutmeg flesh extract: Nutmeg flesh was sorted, cleaned, and cut into small pieces before being blended. The pulp was then pressed and filtered to obtain nutmeg juice. The bioactive compounds with antioxidant properties were extracted from the nutmeg flesh using an extractor.

Production of VCO with nutmeg flesh extract: Coconut flesh was extracted using water and coconut water in a 1:1

ratio to obtain coconut milk. The nutmeg flesh extract was then added at concentrations of 0% (A), 1% (B), 2% (C), and 3% (D) before undergoing fermentation.

The first fermentation stage was conducted in a sealed container naturally, without the addition of microbes, enzymes, or yeast, for 1.5 to 2 hours. This process resulted in the natural separation of protein, oil, and water, after which the water was removed.

The second fermentation stage lasted 10 to 15 hours, producing crude virgin coconut oil (VCO) with a yellowish color. The oil was then purified using a two-stage filtration process to obtain VCO enriched with nutmeg extract (VCO Nutmeg).

The final product was analyzed to determine its medium-chain fatty acids (MCFA) and long-chain fatty acids (LCFA) composition using Gas-Liquid Chromatography (GLC).

Data variables: The analyzed data included fatty acid composition, categorized as:

- **Medium-chain fatty acids (MCFA):** Caproic acid (C6:0), Caprylic acid (C8:0), Capric acid (C10:0), and Lauric acid (C12:0).

- **Long-chain fatty acids (LCFA):** Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), and Linoleic acid (C18:2).

These parameters were measured to determine the overall fatty acid profile of VCO Nutmeg.

Medium-chain fatty acid (MCFA) analysis: MCFA analysis was conducted using Gas-Liquid Chromatography (GLC). Prior to analysis, the VCO samples were prepared as Fatty Acid Methyl Esters (FAME). The method followed AOAC (2012) 969.33 standards.

The gas chromatography conditions were as follows:

- **Column:** Cyanopropyl methylsil (capillary column)
- **Column dimensions:** 60 m length, 0.25 mm internal diameter, 0.25 µm film thickness
- **Carrier gas flow rates:**
  - Nitrogen (N_2): 30 mL/min
  - Hydrogen (H_2): 40 mL/min
  - Air: 400 mL/min

- **Temperature settings:**
  - Injector temperature: **220°C**
  - Detector temperature: **240°C**
  - Column temperature: Programmed

- **Injection parameters:**
  - Split ratio: **1:80**
  - Injection volume: **1 µL**
  - Linear velocity: **23.6 cm/sec**

A 1 µL standard FAME mixture was injected first. Once all peaks were identified, 1 µL of the prepared sample was injected. The retention time and peak profiles of each component were measured and compared with standard retention times to determine the fatty acid composition.

Results and Discussion

The components of fatty acids in Virgin Coconut Oil with the addition of nutmeg fruit flesh extract are presented in Table 1.

Table 1. Components of fatty acids in VCO with nutmeg fruit flesh extract.

Characteristic	Composition (%)	SNI VCO No. 7381: 2008 (%)
Caproic Acid (C6:0)	0.46	ND – 0.7
Caprylic Acid (C8:0)	7.7	4.6 – 10.0
Capric Acid (C-10:0)	6.24	5.0 – 8.0
Lauric Acid (C12:0)	50.59	45.1 – 53.2
Myristic Acid (C14:0)	18.52	16.8 – 21
Palmitic Acid (C16:0)	7.85	7.5 – 10.2
Stearic Acid (C18:0)	2.26	2.0 – 4.0
Linoleic Acid (C18:2)	1.47	1.0 – 2.5

Note: ND = Not detected

The analysis of fatty acid composition confirmed the dominance of medium-chain fatty acids in the VCO samples, particularly lauric acid (C12:0), which accounted for 50.59% of the total fatty acid content. Lauric acid is well-documented for its antimicrobial and immune-enhancing properties, as it is converted to monolaurin in the body, which exhibits antiviral, antibacterial, and antiprotozoal activity. These findings corroborate the results of Gondokesumo (2023), who highlighted the nutritional superiority of MCFAs in energy metabolism and immunity.

The addition of nutmeg extract did not adversely affect the MCFA profile but instead enriched the VCO with bioactive compounds, including phenols, tocopherols, and flavonoids which contribute to its antioxidant and antimicrobial properties (Shafi *et al*., 2025). The significant concentrations of caprylic acid (7.70%) and capric acid (6.24%) observed in the samples align with the Indonesian National Standard (SNI) VCO 7381:2008 quality parameters, ensuring the product's safety and efficacy as a functional food. For a clearer depiction of the fatty acid components, refer to (Fig. 1).

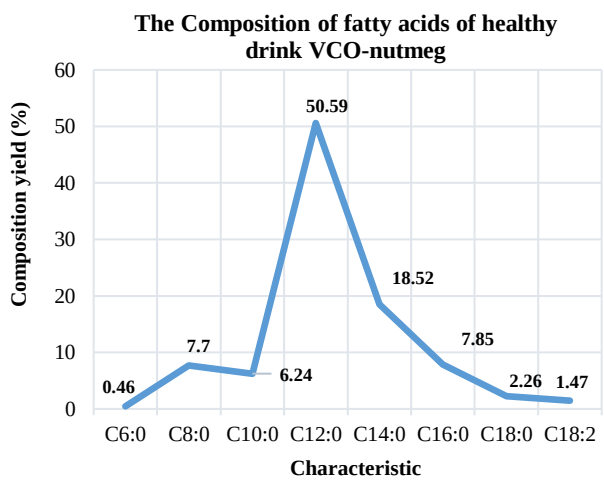

Fig. 1. Fatty acid components of VCO with added spices extract from fruit pulp.

In Table 1 and Figure 1, the results of the analysis of several quality parameters of the Virgin Coconut Oil (VCO) produced according to the treatment are explained. The results for all parameters include a moisture content of 0.13%, an average Free Fatty Acid (FFA) content of 0.09%, a clear color, a characteristic taste and aroma of pure coconut oil (VCO), and a characteristic aroma of nutmeg fruit. The components such as Caproic acid (C6:O), Caprylic acid (C8:O), Capric acid (C10:O), Lauric acid (C12:O), Myristic acid (C14:O), Palmitic acid (C16:O), Stearic acid (C18:O), and Linoleic acid (C18:2) meet the requirements according to the Indonesian National Standard (SNI) VCO Number 7381 of 2008. Lauric acid, a fatty acid component, is a very important compound found in VCO with a minimum percentage of 45.1% according to the SNI VCO, while nutmeg VCO contains a higher lauric acid content of 50.59%.

From the research data, the results show that lauric acid (C12:0) with a percentage of 50.59% is a characteristic component in VCO. Lauric acid is very important because it provides many benefits for the human body. The high content of Medium-Chain Fatty Acids (MCFA) in this research, which is 64.99%, indicates that nutmeg VCO has the potential to offer health benefits, such as enhancing immunity, aiding weight loss, and boosting energy, thus having potential as an immunomodulator.

Long-Chain Fatty Acids (LCFA) and Unsaturated Fatty Acids in this nutmeg VCO research include fatty acids such as Myristic, Palmitic, and Stearic acids, as well as Linoleic acid. Although their percentages are lower than the MCFA, these components also contribute to the nutritional profile of the VCO. Based on the data, nutmeg VCO has a good fatty acid composition, with a high content of MCFA, especially lauric acid.

The ANOVA test results for nutmeg VCO on the composition of Medium-Chain Fatty Acids produced an F-value of 5.21, with a p-value of 0.005 (significant). This indicates a significant difference between the treatments. The addition of nutmeg fruit extract to VCO significantly affected the composition of Medium-Chain Fatty Acids. The treatments of VCO with 2% and 3% nutmeg fruit extract showed significant differences compared to VCO without nutmeg fruit extract. However, the treatment with 1% nutmeg fruit extract did not show a significant difference from VCO without nutmeg fruit extract.

VCO with the addition of nutmeg fruit extract contains spices with phenolic components that are diffused into the VCO, enhancing its quality and activity. According to Pratt & Hudson (1992) in Sipahelut & Rejeki (2021), natural antioxidants found in spices include phenolic or polyphenolic compounds such as tocopherols, coumarins, flavonoids, cinnamic acid derivatives, and organic acids. According to Selonni (2021), antioxidants play an important role in health. Scientific evidence shows that antioxidants can reduce the risk of chronic diseases like cancer and coronary heart disease. Antioxidants are beneficial in treating diseases related directly or indirectly to free radicals. The main characteristic of antioxidants is their ability to capture free radicals. Antioxidants have molecular structures that allow them to donate electrons to free radical molecules without being disturbed and can break the chain reactions of free radicals.

The bioactive compounds in nutmeg fruit extract, as characterized chemically by Dareda *et al*., (2020), show the chemical composition of nutmeg fruit, such as water (9.11%), ash (3.43%), fat (1.81%), protein (4.04%), crude fiber (17.57%), insoluble dietary fiber (48.61%), soluble dietary fiber (1.67%), total dietary fiber (50.28%), hemicellulose (10.72%), cellulose (15.66%), and lignin (19.09%). The antioxidant activity tests show that the free phenolic extract of nutmeg fruit is greater than the bound phenolic extract, and the nitrite scavenging activity of nutmeg fruit shows that the free phenolic extract is greater than the bound phenolic extract.

According to Angeles-Agdeppa *et al*., (2024), VCO supplementation was shown to relieve symptoms and reduce inflammation among COVID-19-positive adults in a single-blind randomized trial, demonstrating its potential as an adjunct therapy against viral infections. These findings reinforce previous studies, such as those by Mandey *et al*., (2021), which highlighted the antimicrobial benefits of lauric acid in enhancing immunity and supporting recovery in COVID-19 patients. Lauric acid, according to previous research by Mandey *et al*., (2021), is capable of killing pathogenic microbes such as viruses, bacteria, fungi, etc., and can enhance immunity, improve brain function, and memory in patients infected with Covid-19, thus aiding in their recovery. The antimicrobial properties of nutmeg VCO align with findings by Rahmadi *et al*., (2013), who reported enhanced antibacterial activity in VCO fermented with lactic acid bacteria. The lauric acid content in the produced VCO is 50.59%, while in breast milk (ASI) it is 6.2%. Thus, the antimicrobial properties of this VCO product are very high. When lauric acid is consumed and enters the human body, it becomes monolaurin. The synergy of fatty acids and monoglycerides is very effective in disrupting and killing the cell walls of pathogenic microbes. VCO also contains vitamin E (antioxidants), which is beneficial for health because it contains medium-chain fatty acids (Pramitha & Karta, 2021).

According to Nasir & Marwprattati (2022) Virgin Coconut Oil (VCO) and nutmeg fruit extract are both known for their numerous health benefits. The combination of both has the potential to provide a synergistic immunomodulatory effect.

The Immunomodulatory potential of VCO, particularly with the presence of lauric acid, means that nutmeg VCO has antiviral, antibacterial, and antifungal properties. Lauric acid can enhance the immune system by disrupting the membranes of pathogenic cells, increasing the activity of immune cells like macrophages and lymphocytes, and stimulating the production of cytokines (messenger proteins in the immune system). With the presence of antioxidants like vitamin E in VCO, nutmeg VCO is believed to protect immune cells from damage caused by free radicals.

According to Syahriani *et al*., (2023), VCO is processed by pressing without heating or with heating not exceeding 60°C and is safe for human consumption. Virgin coconut oil (VCO), like other vegetable oils, contains natural antioxidants such as tocopherols, which protect the oil from oxidation. Additionally, the main components of

VCO consist of Medium-Chain Triglycerides (MCTs), which are resistant to peroxidation. These fatty acids are different from those found in animal fats, which contain long-chain saturated fatty acids. VCO products have no color, are free from sediment, have a fresh natural coconut aroma, and are free from rancid odors or tastes (Anon., 2009). The quality standards for good VCO have been specified in the Indonesian National Standard (SNI) VCO Number 7381:2008.

The Medium-Chain Fatty Acids in VCO are known for their nutritional and health benefits and are recognized as a multipurpose nutritional supplement. These components differ from other triglycerides because each fat molecule has a chain length between C6, C8, C10, and C12.

The uniqueness of the Medium-Chain Fatty Acids in VCO, combined with the addition of nutmeg fruit extract, has bioactive potential. Nutmeg also has promising prospects as a phytopharmaceutical in the future, in addition to being beneficial in obstetrics, pharmacy, including essential oils, and other substances containing antioxidants (Kamelia *et al*., 2018). Nutmeg's bioactivity as an antioxidant, antibacterial, anti-inflammatory, and antifungal when combined with VCO offers significant health benefits.

The fatty acids in Virgin Coconut Oil (VCO), such as lauric acid (a saturated fatty acid), are the dominant fatty acids and have similar properties to those found in breast milk. When consumed, these can be converted into monolaurin, which functions as a supplement to prevent degenerative diseases and diseases caused by viruses and bacteria. This product is also effective, according to research by Mandey *et al*., (2021), in improving body health, boosting immunity, as lauric acid also acts as an antibiotic, antiviral, antibacterial, and antiprotozoa agent and can improve the body's metabolism. Previous research by Mandey *et al*. (2021), through the Covid-19 Research Consortium of the Ministry of Research and Technology BRIN RI, selected this VCO product as an Indonesian Innovation Product at the 2020 Innovation Exhibition. It was diffused by the Ministry of Research and Technology BRIN RI and has been provided to Covid-19 patients at several hospitals in Indonesia.

In previous research (Mandey *et al*., 2021), this product was of good quality according to the Indonesian SNI VCO Number 7381 of 2008. However, to further enhance its immunological function, this VCO product has been supplemented with bioactive compounds from nutmeg fruit extract as a functional food source that is healthy and contains bioactive compounds beneficial to health and functions as an immunomodulator. Mandey *et al*., (2022) investigated the positioning strategy, trust, and brand image of VCO products, highlighting their impact on purchase intentions in MSME settings. The enhanced quality and bioactivity of nutmeg VCO, as demonstrated in this study, could strengthen its market positioning as a premium functional food, appealing to health-conscious consumers. The combination of scientific validation and market appeal underscores the potential of nutmeg VCO for both health promotion and commercial success.

Nutmeg has great prospects as a promising phytopharmaceutical in the future, in addition to being beneficial in the fields of obstetrics, pharmacy, essential oils, and other substances containing antioxidants (Kamelia *et al*., 2018). Nutmeg's bioactivity as an antioxidant, antibacterial, anti-inflammatory, and antifungal agent. The chemical components of nutmeg fruit that contribute to its antibacterial properties include flavonoids, phenols, saponins, and tannins from the outer shell, as well as phenols, terpenoids, flavonoids, and alkaloids from the seeds. The chemical components responsible for its antioxidant activity in nutmeg seeds include 5-Octadecanoic acid, myristicin, phenol, terpineol, and 9-octadecenoic acid, while in the nutmeg fruit flesh, they include alkaloids and vitamin C. The chemical components that contribute to its antifungal properties in nutmeg seeds include monoterpenes, flavonoids, and alkaloids, while in the fruit flesh, they include flavonoids, saponins, and alkaloids (Suloi, 2021).

Conclusion

The results of this research indicate that:

1. The addition of *nutmeg* fruit extract to Virgin Coconut Oil (VCO) has a significant effect on the composition of medium-chain fatty acids. The addition of *nutmeg* fruit extract at concentrations of 2% and 3% can increase the lauric acid (C12:0) content. The fatty acids detected in VCO with the addition of *nutmeg* fruit extract (VCO *Nutmeg*) include: Caproic acid (C6:0) at 0.46%, Caprylic acid (C8:0) at 7.70%, Capric acid (C10:0) at 6.24%, Lauric acid at 50.59%, Myristic acid (C14:0) at 18.52%, Palmitic acid (C16:0) at 7.85%, Stearic acid (C18:0) at 2.26%, and Linoleic acid (C18:2) at 1.47%. This finding suggests that VCO *Nutmeg* has potential as a health-promoting product and could lead to the development of functional food products that synergistically enhance immune function and general health.

2. Virgin Coconut Oil (VCO) and *nutmeg* fruit extract both have numerous health benefits. Their combination holds the potential for a synergistic immunomodulatory effect, which may contribute to the prevention of infections, improved immune function, and metabolic health. Future research could further explore the bioactive compounds from *nutmeg* and their synergistic effects with VCO, as well as assess the potential for large-scale production and clinical applications, particularly in functional foods and health supplements.

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