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Research Article

Bromelain-Extracted of Virgin Coconut Oil: Physical and Chemical Stability in Different Temperature During the Storage

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Abstract

The bromelain-enzymatic reaction is applied in virgin coconut oil (VCO) production. Hydrolysis of the emulator by bromelain enzyme in coconut milk during fermentation maximized further the separation of oil and water. In the higher demand for VCO in many industries, the VCO stability during transportation and storage must be evaluated. The research aims to evaluate the physical and chemical stability of the effect of temperature in the storage. VCO's physical and chemical stability was evaluated under two different temperature and storage periods: an elevated temperature of 50°C for 10 days and room temperature (27-30°C) for 50 days. The storage was conducted in a clear glass bottle. The evaluation was based on physical and chemical stability tests before and after storage, including organoleptic, pH changes, density, viscosity, acid number, peroxide number, and saponification number. Based on the data, the quality of the VCO after storing at 50°C for 10 days and 27-30°C for 50 days was found to be changed for pH, specific gravity, viscosity, acid number, peroxide number, saponification number, while for the appearance was found to be no changes. The VCO was very sensitive to environmental effects. Therefore, it is necessary to find the best storage chamber and temperature for stabilizing the VCO.

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INTRODUCTION

Indonesia, a Southeast Asian nation characterized by its tropical climate, boasts a rich diversity of plant life. Among these readily available plants is the coconut (*Cocos nucifera*)[1]. Notably, all parts of the *C. nucifera* palm find utility, from the leaves and roots to the stems and fruit[2]. *Cocos nucifera* fruit, in particular, serves as the primary source of virgin coconut oil (VCO)[3,4]. Virgin coconut oil, renowned for its applications in both daily consumption and pharmaceutical contexts, undergoes various processing techniques to yield a product characterized by low water content, minimal free fatty acids, a clear appearance, a distinct aroma, and a shelf life exceeding 12 months[5-7]. Virgin coconut oil production utilizes various methods, including traditional, enzymatic, thermal, acidification, centrifugation, and inducement techniques[8-10] Among these, the enzymatic process offers distinct advantages.

Bromelain, an enzyme extracted from pineapples (*Ananas comosus*), plays a crucial role in this method. It accelerates the breakdown of the *C. nucifera* milk emulsion system by hydrolyzing peptide bonds, leading to oil separation[11,12]. *Ananas comosus*, a widely cultivated tropical fruit, is a rich source of bromelain, particularly in its yellow flesh[13]. While *A. comosus* are known for their high water content (approximately 90%) and essential minerals like potassium, calcium, iodine, sulfur, and chlorine[14], bromelain emerges as the key component for VCO production due to its efficient oil separation properties.

Virgin coconut oil has gained significant popularity due to its perceived health benefits. Consequently, ensuring the consistent delivery of high-quality VCO to consumers is paramount[15]. However, the quality of VCO can deteriorate during

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transportation and storage[16]. During transport, exposure to elevated temperatures exceeding 50°C can occur within vehicles[17]. Similarly, storage conditions are often inconsistent, leading to temperature fluctuations[18]. Several studies have documented that unstable storage temperatures contribute to a decline in VCO quality[19,20]. Therefore, research investigating the stability of VCO produced using the bromelain enzymatic method is crucial to establishing accurate expiration dates. This research should encompass a battery of stability parameters, including organoleptic tests (color, odor, and taste), changes in pH, specific gravity, viscosity, acid number, peroxide number, and saponification number.

MATERIALS AND METHODS

Materials

The instruments used include equipment for VCO production and quality analysis. Virgin coconut oil production equipment typically included blenders, spatulas, chambers, analytical balances, measuring cylinders, and clear glass bottles. Quality analysis equipment used in this study included Erlenmeyer flasks, beakers, measuring flasks, conical flasks, porcelain cups, glass funnels, weighing bottles, analytical balances, stirrers, ovens, pH meters, pycnometers, cooling baths, and Brookfield viscometers.

Cocos nucifera and *A. comosus* were purchased from the Gamping traditional market in Yogyakarta, Indonesia. The scientific identification of these raw materials was confirmed by the Biological Education Laboratory, Faculty of Science and Applied Technology, Universitas Ahmad Dahlan, Yogyakarta (reference numbers: 454/Lab.Bio/B/XI/2023 for *A. comosus* and 474/Lab.Bio/B/XII/2023 for *C. nucifera*). For the quality analysis of VCO, analytical grade chemicals were used without further purification. These chemicals included distilled water, 95% ethanol, standardized NaOH, oxalic acid, standardized KOH, 0.5 N alcoholic KOH, phenolphthalein indicator, HCl, acetic acid, chloroform, sodium thiosulfate, potassium dichromate, and starch powder. All analytical grade chemicals were purchased from Merck, Germany.

Methods

Preparation of VCO

Raw materials were weighed according to the experimental design. Grated *C. nucifera* flesh was added to distilled water at a 1 : 1 ratio. The mixture was then homogenized and filtered to obtain *C. nucifera* milk. The *C. nucifera* milk was stored in a transparent container at room temperature to allow for separation into cream and skim layers. After separation, the top layer (*C. nucifera* cream) was carefully collected using a sterile pipette to avoid contamination from the lower skim layer (water). Fresh *A. comosus* fruits were peeled, washed, and blended using a sanitized blender. The homogenized mixture was then filtered to obtain clarified *A. comosus* juice.

Cocos nucifera cream and *A. comosus* juice were mixed at a 10 : 1 ratio and homogenized to ensure a uniform mixture. The homogenized mixture was stored in a transparent container at room temperature for 24 hours to allow for phase separation into three distinct layers: water (bottom layer), VCO (middle layer), and residue (top layer). The VCO layer was carefully separated using a sterile straw inserted into the middle of the container. The collected VCO was then analyzed for fatty acid composition using gas chromatography-mass spectrometry (GC-MS). Fatty acid composition analysis was performed using a GC-MS system equipped with a 30-meter DB-5MS column. Helium gas served as the mobile phase at a flow rate of 33.2 mL/minute. The injection port temperature was set to 300°C, and the initial column temperature was maintained at 70°C.

Data analysis

This study evaluated the physical and chemical stability of VCO in two storage conditions: room temperature (27-30°C) for 50 days and high temperature (50°C) for 10 days. The stability test was determined through various tests, including:

1. Organoleptic evaluation: This involved assessing color, odor, and taste of the VCO samples.
2. pH measurement: A pH meter was used to measure changes in the acidity or alkalinity of the VCO during storage.
3. Specific gravity determination: A pycnometer was employed to measure the density of the VCO samples.
4. Viscosity measurement: A Brookfield viscometer was used to assess changes in the viscosity of the VCO over time.
5. Chemical analysis: This included determination of acid number, peroxide number, and saponification number, following standard methods[21]. It's important to note that all analyses were performed at room temperature.

RESULTS AND DISCUSSION

Our previous study[22] has demonstrated that incorporating bromelain extract from *A. comosus* juice into the processing of VCO can significantly increase yield. In these studies, 1000 mL of *C. nucifera* milk yielded an average of 300 mL of VCO when combined with 100 mL of *A. comosus* juice. The bromelain enzyme in *A. comosus* juice is hypothesized to hydrolyze proteins within the *C. nucifera* milk during fermentation. This process disrupts the emulsion of oil and water in the *C. nucifera* milk, potentially maximizing oil extraction and leading to a higher VCO yield compared to traditional methods without bromelain supplementation.

Gas chromatography is a well-established technique for analyzing fatty acid methyl esters (FAMEs) in oils. It separates components in a mixture based on their interactions with the mobile (gas carrier) and stationary phases within the GC column[23]. The method offers rapid separation, typically within seconds, as the gas flow carries vaporized samples through the column. The separated components are detected, and a chromatogram (a graphical representation of detector response versus time) is generated. The specific characteristics of the GC instrument, including column dimensions (length and diameter) and operating conditions (gas flow rate and oven temperature), influence the separation and retention times of the FAMEs[24].

Our GC analysis of the VCO sample identified several fatty acids. These included caproic acid (retention time: 7.3 minutes), caprylic acid (14.2 minutes), capric acid (20.6 minutes), lauric acid (26.3 minutes, highest peak), myristic acid (31.2 minutes), palmitic acid (35.7 minutes), linoleic acid (39.1 minutes), oleic acid (39.2 minutes), and stearic acid (39.7 minutes). The chromatogram is presented in **Figure 1**, and **Table I** summarizes the identified fatty acids and their corresponding retention times. As evident from the highest peak in **Figure 1** (peak number 4), lauric acid was the most abundant fatty acid in the VCO sample. The peak height in a chromatogram generally corresponds to the relative concentration of the component in the sample. This finding aligns with Indonesian National Standard (*Standar Nasional Indonesia*; SNI) 7381-2022 for VCO, which specify lauric acid as the predominant fatty acid[21].

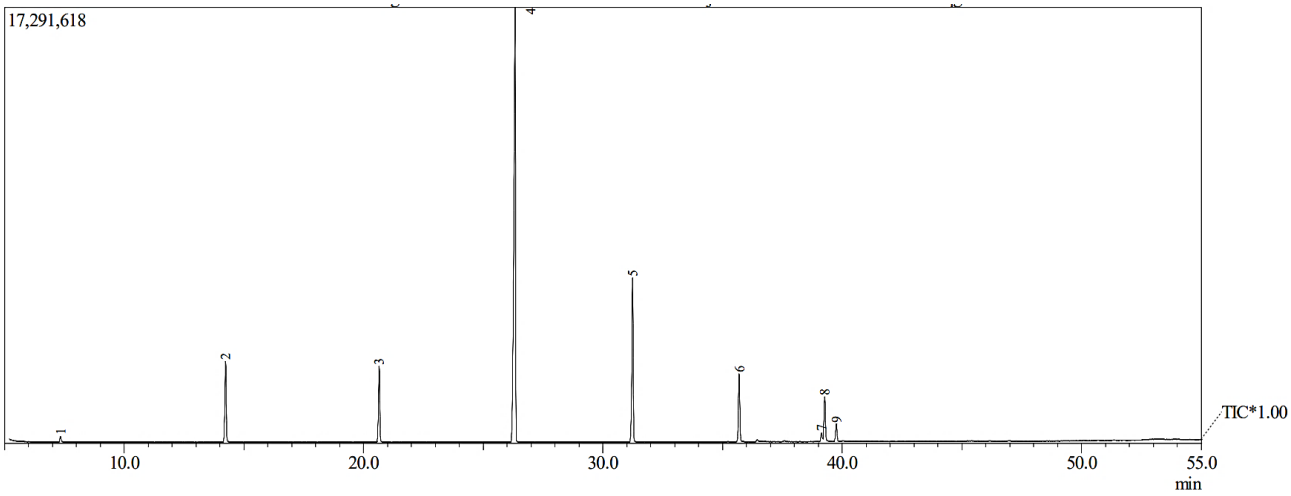

Figure 1. GC-MS Chromatogram of bromelain-extracted VCO.

Table I. Summarized fatty acids of bromelain-extracted VCO.

Peak	Rt (minutes)	Fatty acid
1	7.3	Caproic acid
2	14.2	Caprylic acid
3	20.6	Capric acid
4	26.3	Lauric acid
5	31.2	Myristic acid
6	35.7	Palmitic acid
7	39.1	Linoleic acid
8	39.2	Oleic acid
9	39.7	Stearic acid

The stability of the emulgel formulations was assessed through organoleptic evaluation, pH measurement, specific gravity determination, viscosity measurement, acid value testing, peroxide value testing, and saponification value testing. **Table II** summarizes the changes observed in these stability indicators during storage at room temperature (25-27°C) for 50 days and at 50°C for 10 days. Organoleptic evaluation was performed to assess the color, odor, and taste of the VCO samples. Fresh VCO is characterized by a clear yellowish color, a pleasant *C. nucifera* odor, and a savory *C. nucifera* taste[21]. The VCO produced in this study maintained these characteristics throughout the storage period, with no significant changes observed in color, odor, or taste between the VCO before treatment and the samples stored for ten days at 50°C, 50 days at room temperature (27-30°C), or both. The resulting taste still tastes savory *C. nucifera* without a stale taste even though it has been in extreme temperatures and has been stored for a long time. These findings indicate that the VCO formulation exhibits good stability in terms of organoleptic properties.

The pH of VCO was measured to assess its stability during storage. A pH meter was used to determine the initial pH of VCO, which was 5.857. Virgin coconut oil samples stored at 50°C for 10 days exhibited a slight decrease in pH to 5.590. Similarly, samples stored at room temperature for 50 days showed a decrease in pH to 5.598. These observations suggest a potential decrease in VCO stability during storage, particularly at elevated temperatures. The decrease in pH may be attributed to the hydrolysis of triglycerides in VCO into free fatty acids, which are acidic in nature[20]. Further studies are needed to investigate the extent of free fatty acid formation and its impact on other quality parameters of VCO during storage.

Specific gravity, defined as the ratio of a substance's density to that of water at the same temperature, is an indicator of oil quality. The standard range for VCO specific gravity is 0.9150-0.9244 g/cm^3[21]. Our analysis revealed a specific gravity of 0.9235 g/cm^3 for the VCO before treatment. This value falls within the established acceptable range. However, a significant decrease in specific gravity was observed after storage, with values of 0.8939 and 0.9023 g/cm^3 for samples stored at 50°C for 10 days and at room temperature for 50 days, respectively. This decrease suggests potential instability during storage. The observed decrease in specific gravity during storage aligns with the notion that hydrolysis reactions are accelerated at higher temperatures[25]. These reactions break down triglycerides, releasing glycerol and free fatty acids. Glycerol has a higher density than most common fatty acids found in VCO. Therefore, as hydrolysis progresses, the overall density of the oil decreases, leading to a lower specific gravity[26]. This highlights the importance of proper storage conditions to maintain VCO quality.

Viscosity, a measure of a fluid's resistance to flow, was determined using a Brookfield viscometer. This instrument utilizes a rotating spindle at a specific speed to measure the resistance of the sample[27]. The appropriate spindle selection depends on the sample's viscosity; higher spindle numbers correspond to smaller physical dimensions and are suitable for testing thicker, more viscous materials[28]. In our study, three key factors influenced viscosity measurements: spindle speed (RPM), torque range (scale), and spindle number[27]. The viscosity of the untreated VCO was 13.8 cP. VCO stored at 50°C for 10 days and at room temperature for 50 days exhibited viscosities of 13.6 and 13.5 cP, respectively. These observations suggest a decrease in VCO viscosity during storage, potentially indicating a form of instability. The observed decrease in viscosity may be attributed to the hydrolysis process occurring during storage.

The acid number, expressed as mg of base (NaOH) required to neutralize free fatty acids present in 1 g of oil, serves as an indicator of oil quality. Higher acid numbers reflect increased free fatty acid content, which can arise from hydrolysis, heating, enzymatic reactions, or physical processing[19]. In this study, untreated VCO exhibited an acid number of 0.48 mg NaOH/g. Notably, storage at elevated (50°C for 10 days) and room temperatures (for 50 days) resulted in modest increases in acid number to 0.72 and 0.56 mg NaOH/g, respectively. While these increases suggest a slight decrease in VCO stability during storage, the overall changes were relatively small. These findings are consistent with previous studies[29,30], which report that VCO generally exhibits good stability due to its high content of lauric acid, a saturated fatty acid resistant to hydrolysis. However, prolonged storage at elevated temperatures can accelerate hydrolysis, leading to a gradual increase in free fatty acid content and acid number.

Peroxide number is a well-established indicator of oil damage caused by oxidation. It reflects the concentration of peroxides formed during the oxidation process, expressed as milliequivalents (meq) of active oxygen per kg of oil. As observed in this study, an increase in peroxide number indicates ongoing oxidation within the oil. The free radical chain reaction initiated by the interaction between oxygen and double bonds in the fatty acids progressively generates peroxides[31,32]. However, a low peroxide number doesn't necessarily imply minimal oxidation. The fatty acid composition of the oil also influences its

peroxide number[33]. The peroxide number of untreated VCO in our study was 1.8 meq O_2/kg. Storage at 50°C for 10 days increased the peroxide number to 5.6 meq O_2/kg, and storage at room temperature for 50 days resulted in a peroxide number of 4.8 meq O_2/kg. Notably, both storage conditions (room temperature for 50 days and 50°C for 10 days) yielded peroxide number values exceeding SNI 7381-2022 for VCO (20 meq O_2/kg)[21]. This observation aligns with the established notion that higher storage temperatures accelerate oil oxidation, leading to increased peroxide numbers[34]. Our findings suggest that storing VCO in a cool environment is crucial to maintaining its quality and preventing excessive oxidation.

The saponification number, which reflects the amount of KOH required to saponify 1 g of oil, is inversely proportional to the oil's molecular weight. This means that oils with higher saponification numbers contain shorter fatty acid chains. Shorter chain fatty acids are generally considered more resistant to oxidation and hydrolysis, contributing to increased oil stability and reduced susceptibility to rancidity[35]. Our study investigated the changes in the saponification number of VCO following storage at different temperatures. The initial saponification number of the VCO was 258.06 mg KOH/g. After storage at 50°C for 10 days, the saponification number decreased to 246.84 mg KOH/g. Similarly, storage at room temperature (27-30°C) for 50 days resulted in a decrease in the saponification number to 240.66 mg KOH/g. These observations are consistent with the established relationship between saponification number and oil stability. The decrease in saponification number following storage suggests a potential breakdown of triglycerides in the VCO, likely due to hydrolysis or oxidation reactions. This breakdown could result in the formation of shorter chain fatty acids, contributing to the observed decrease in saponification number[36]. However, further investigation is needed to confirm the specific nature of the ongoing chemical reactions during storage.

Table II. Summarized value of stability test.

Description	Value		
	Initial	**Storage at 25-27°C for 50 days**	**Storage at 50°C for 10 days**
Smell, taste, and color	Specific *C. nucifera* smell, oily, and yellowish white	Specific *C. nucifera* smell, oily, and yellowish white	Specific *C. nucifera* smell, oily, and yellowish white
pH	5.857±0.024	5.598±0.011	5.59±0.01
Specific gravity (g/cm^3)	0.924±0.013	0.902±0.03	0.894±0.024
Viscosity (Cp)	13.8±0.076	13.6±0.038	13.6±0.076
Acid number (mg NaOH/g)	0.48±0.01	0.56±0.005	0.72±0.015
Peroxide number (meq O_2/kg)	1.8±0.017	4.8±0.05	5.6±0.066
Saponification number (mg KOH/g)	258.06±0.106	240.66±0.131	246.84±0.045

CONCLUSION

This study investigated the influence of storage temperature and duration on the quality of VCO. The results revealed significant changes (pH, specific gravity, viscosity, acid number, peroxide number, and saponification number) in VCO stored at 50°C for 10 days compared to those stored at room temperature (27-30°C) for 50 days. Notably, the appearance of the VCO remained unchanged across all storage conditions. These findings suggest that VCO is highly sensitive to elevated temperatures. Further research is warranted to identify optimal storage conditions that effectively preserve the quality of VCO.

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AUTHORS' CONTRIBUTION

Conceptualization: Sabtanti Harimurti
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Formal analysis: Sabtanti Harimurti, Hasna Fadia Sari
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Software: Sabtanti Harimurti, Hasna Fadia Sari
Supervision: Dyani Primasari Sukamdi, Hari Widada
Validation: Hari Widada, Azura Amid
Visualization: Sabtanti Harimurti
Writing - original draft: Sabtanti Harimurti
Writing - review & editing: Hari Widada, Azura Amid

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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