

Physicochemical Stability of Virgin Coconut Oil (VCO) Produced using Bromelain Enzyme from Pineapple Waste (*Ananas comosus* L.)

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Abstract. Virgin coconut oil (VCO) is a vegetable oil extracted from fresh coconut meat, after undergoing heating at a maximum temperature of 60°C or the addition of chemicals (without heating) to ensure that the oil structure does not change and its compound content is preserved. High-quality VCO must meet the requirements set by the Indonesian National Standards Agency through the Indonesian National Standards (SNI). Enzymatic methods are one of the most commonly used methods for producing VCO, as they can increase the oil yield extracted from coconut cream. Bromelain enzymes can be produced by pineapple plants (*Ananas comosus* L.). The objective of this study was to determine the physicochemical stability of enzymatically produced VCO from pineapple extracts to obtain a quality that complies with SNI standards. VCO is made from coconut cream derived from coconut flesh, and bromelain enzyme is extracted from pineapple plants (crown, leaves, peel, and stem). The physicochemical stability of VCO includes organoleptic testing, free fatty acid content, moisture content, and peroxide value. Testing was conducted at room temperature (for 50 days) and 65°C (for 10 days). The physicochemical stability tests yielded varied results, depending on the plant parts used.

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1 Introduction

Virgin coconut oil (VCO) is a plant-based oil extracted from fresh coconut meat using gentle heat (up to 60°C) or chemical methods without heat, preserving its natural structure and beneficial compounds by avoiding evaporation, oxidation, hydrogenation, and other chemical changes [1]; [2]. VCO contains medium-chain fatty acids like lauric and caprylic acid, offering various health benefits, including managing hypercholesterolemia, diabetes, hypertension, and exhibiting antiviral and antibacterial properties [1]; [2]; [3]; [4]. In addition to lauric and caprylic acid, VCO contains tocopherols and beta-carotene, which act as antioxidants. These compounds help prevent premature aging, osteoporosis, and support vitality, while also protecting VCO from oxidation [5].

VCO is considered high quality if it meets the Indonesian National Standard (SNI) set by the National Standardisation Agency. Organoleptically, it should be pale yellow or colourless, tasteless, and have a distinct coconut aroma. It typically contains no more than 0.2% free fatty acids (as lauric acid), 0.2% moisture, and a peroxide value below 2 mEq/kg [6]. Oil quality can be assessed by its appearance and peroxide value—the lower the peroxide value, the higher the quality. High temperatures during VCO production accelerate oxidation, leading to rancid odours [7]. VCO characteristics like pH, yield, free fatty acids, moisture content, and clarity are greatly affected by incubation time during production [8].

VCO can be extracted through physical (centrifugation and low heat), chemical (acidification), or enzymatic (protein-degrading enzymes) methods [9]. The enzymatic method is widely used for its ability to increase the oil yield from coconut milk cream [10]; [11]. Bromelain is commonly used in VCO production to hydrolyse proteins, promoting the separation of oil and water in coconut milk emulsion [11]; [12].

Bromelain, a protease enzyme found in various parts of the pineapple plant (*Ananas comosus* L.) including the flesh, crown, peel, leaves, and stem can catalyse protein hydrolysis and enhance VCO yield. While the flesh is commonly used, other discarded parts also contain bromelain and can be utilized in VCO production [12]; [13]. Enzymatically produced VCO using bromelain from pineapple extracts meets SNI aroma standards, retaining the characteristic coconut scent despite a slightly watery texture. Visually, only VCO from stem extract is colourless, while that from leaves, crown, and rind appears greenish.

This study aims to assess the physicochemical stability of enzymatically produced VCO from pineapple extract and its compliance with SNI standards, by examining the effects of varying storage temperatures and durations.

2 Methods

This study used pure coconut milk from coconut flesh and pineapple plant extracts (crown, leaves, peel, and stem). Pineapples were sourced from the Bagong plantation in Bantul and identified at the Biology Laboratory, Ahmad Dahlan University (Ref:

454/Lab.Bio/B/XI/2023). Coconuts were obtained from Gamping Central Fruit Market and identified at the same lab (Ref: 474/Lab.Bio/B/XII/2023).

Other materials used include standard VCO (Ikan Dorang[®]); distilled water (PT. Brataco[®]); ethanol (PT. Brataco[®]); p.a. methanol (Supelco[®]); n-hexane p.a. (Supelco[®]); chloroform p.a. (Supelco[®]); cassava starch; PP indicator (Alfa Kimia[®]); and 0.1 N NaOH (Alfa Kimia[®]).

2.1 Coconut cream

Coconut flesh is washed, grated, and mixed with clean water at a 1:1 w/v ratio. The mixture is squeezed and filtered to obtain coconut milk, which is left to settle for 2 hours until cream and skim layers separate. The cream is then collected for VCO production [14].

2.2 Pineapple extract

Each pineapple plant part was washed, chopped, and ground into powder, then extracted with distilled water (1:1 w/v). The mixture was squeezed, filtered, and the extract collected and measured [14].

2.3 VCO

Coconut cream was mixed with pineapple extract at a 1:1 v/v ratio and stirred until homogeneous. The mixture was left for 24 hours at room temperature to separate, and the resulting oil (VCO) was collected and measured [14].

2.4 Physicochemical stability

2.4.1 Organoleptic test

Organoleptic testing of VCO's odour, colour (clarity), and flavour was scored on a 1-5 scale (see Table 1) at room temperature (50 days) and 65°C (10 days).

Table 1. Organoleptic scoring.

Parameter	Scoring criteria
Odour	1 : very rancid
	2 : rancid
	3 : slightly rancid
	4 : not rancid
	5 : not at all rancid
Colour	1 : very unclear
	2 : unclear
	3 : slightly unclear
	4 : clear
	5 : very clear
Flavour	1 : very rancid

Parameter	Scoring criteria
	2 : rancid
	3 : slightly rancid
	4 : not rancid
	5 : not at all rancid

2.4.2 Free fatty acid

VCO is weighed, neutralized with heated 96% ethanol, and mixed with a PP indicator. It is then titrated with standardized 0.1 N NaOH until a stable pink colour appears for 30 seconds. The free fatty acid content (%FFA) is calculated using Equation 1.

$$\% FFA = \frac{M.A.N}{10.G} \cdot 100\% \quad (1)$$

2.4.3 Peroxide value

VCO is weighed and mixed with a peroxide solution (20 mL acetic acid, 55 mL chloroform). Potassium iodide is added and shaken for 1 minute until yellow. After adding distilled water, the solution is titrated with 0.01 N sodium thiosulfate using starch indicator, stopping when the solution turns pale yellow [15]. The peroxide number can be calculated using the following equation 2.

$$\text{Peroxide value} = \frac{V.N.1000}{W} \quad (2)$$

2.4.4 Water content

VCO is weighed in a porcelain dish, dried in an oven at 105°C for 4–5 hours until weight is constant, then cooled in a desiccator for 15–30 minutes before final weighing. Moisture content is calculated using Equation 3.

$$\text{Water content} = \frac{W_0 - W_t}{W_0} \cdot 100\% \quad (3)$$

3 Results and discussion

VCO from crowns, leaves, and peels appeared pale yellow and clear, while stem-derived VCO matched the transparent clarity of the market standard (Figs. 1 and 2). All samples met SNI requirements. Organoleptic tests of the produced VCO, compared to the standard, were conducted at 25°C for 50 days and 65°C for 10 days. Results are shown in Tables 2 and 3. At room temperature, VCO odour remained non-rancid, but by day 50, VCO from leaves and stems developed a rancid smell due to fat hydrolysis. Unlike the centrifuge-purified standard, VCO treated with zeolites may retain some water, which promotes hydrolysis and rancidity. The colour of VCO tested at both room temperature and 65°C remained within SNI standards.

After organoleptic testing, VCO and the standard were analysed for free fatty acid (FFA) content, measured by the mg of NaOH needed to neutralize FFA per gram of oil. High FFA levels result from hydrolysis, which is accelerated by heat, water, acidity, and enzymes. Crown VCO samples had free fatty acid levels above 0.2% at both room temperature and 65°C, failing SNI standards. This was due to extended storage and high temperatures

accelerating oil hydrolysis. Standard VCO met SNI free fatty acid limits at room temperature up to day 30 but exceeded them by days 40 and 50 (Fig. 3). At 65°C, levels remained below 0.2%, meeting SNI standards (Fig. 4).

Table 2. An organoleptic test of VCO and standard at room temperature.

Parts of the plant	Parameter	VCO						Standard					
		Days						Days					
		0	10	20	30	40	50	0	10	20	30	40	50
Crown	Odour	4	4	4	4	4	4	5	5	5	5	5	5
	Colour	4	4	4	4	4	4	5	5	5	5	5	5
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5
Leaves	Odour	5	5	5	5	4	3	5	5	5	5	5	5
	Colour	5	5	5	5	5	5	5	5	5	5	5	5
	Flavour	4	4	4	4	4	3	5	5	5	5	5	5
Peel	Odour	4	4	4	4	4	4	5	5	5	5	5	5
	Colour	5	5	5	5	5	5	5	5	5	5	5	5
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5
Stem	Odour	5	4	4	4	3	3	5	5	5	5	5	5
	Colour	5	5	5	5	5	5	5	5	5	5	5	5
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5

Table 3. An organoleptic test of VCO and standard at 65°C.

Parts of the plant	Parameter	VCO							Standard					
		Days							Days					
		0	2	4	6	8	10	0	2	4	6	8	10	
Crown	Odour	4	4	4	4	4	4	5	5	5	5	5	5	
	Colour	4	4	4	4	4	4	5	5	5	5	5	5	
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5	
Leaves	Odour	5	5	5	5	5	4	5	5	5	5	5	5	
	Colour	5	5	5	5	5	5	5	5	5	5	5	5	

Parts of the plant	Parameter	VCO						Standard					
		Days						Days					
		0	2	4	6	8	10	0	2	4	6	8	10
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5
Peel	Odour	4	4	4	4	4	4	5	5	5	5	5	5
	Colour	5	5	5	5	5	5	5	5	5	5	5	5
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5
Stem	Odour	5	4	4	4	4	4	5	5	5	5	5	5
	Colour	5	5	5	5	4	4	5	5	5	5	5	5
	Flavour	4	4	4	4	4	4	5	5	5	5	5	5

The free fatty acid results for leaf VCO differed slightly. At both room temperature and 65°C, only day 0 met SNI standards (Figs. 3 and 4). In contrast, the standard VCO met SNI at 65°C throughout the testing period (Fig. 4). At room temperature, the standard complied until day 30 but failed on days 40 and 50 (Fig. 3). For peel VCO, none of the samples met SNI requirements at either temperature (Figs. 3 and 4). Stem VCO showed similar results, failing to meet standards under both conditions (Figs. 3 and 4). The standard’s relatively low free fatty acid levels are due to thorough standardization, including purification, centrifugation, and filtration, resulting in lower water content and better quality compared to sample VCO.

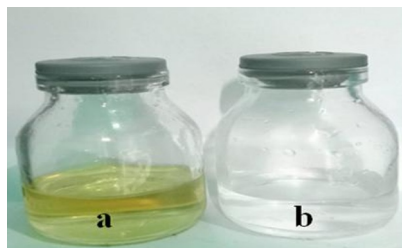


Fig. 1. Enzymatic VCO crowns, leaves, and peels (a) and standard (b).

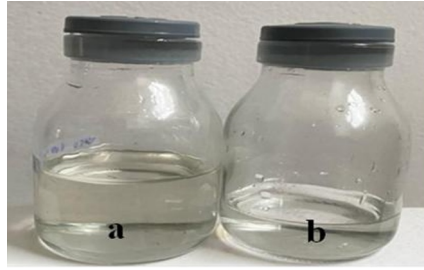


Fig. 2. Enzymatic stem VCO (a) and standard (b).

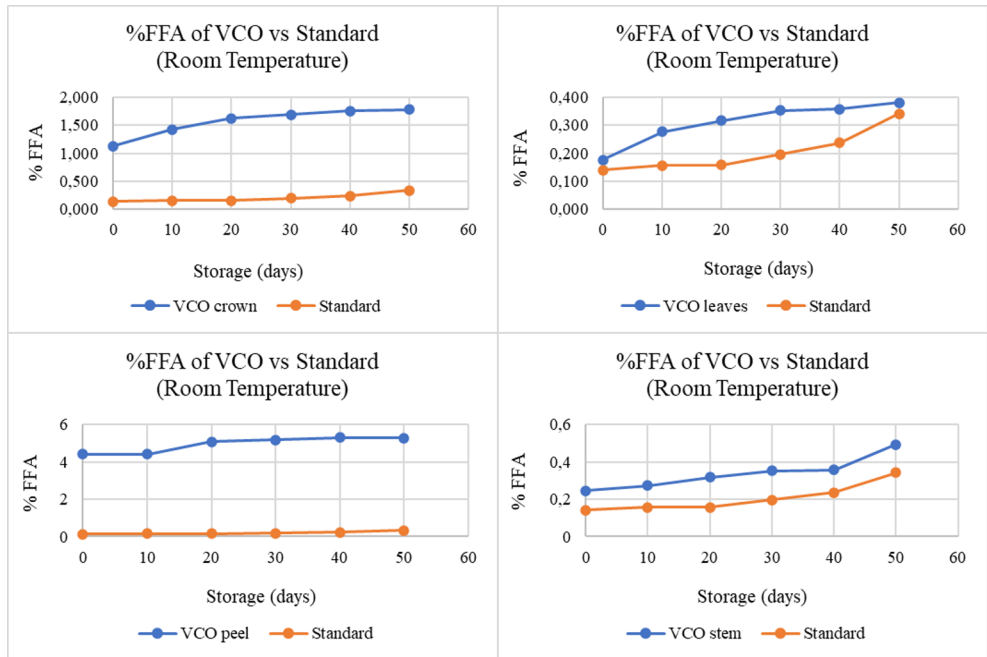


Fig. 3. Free fatty acid of VCO and standard at room temperature.

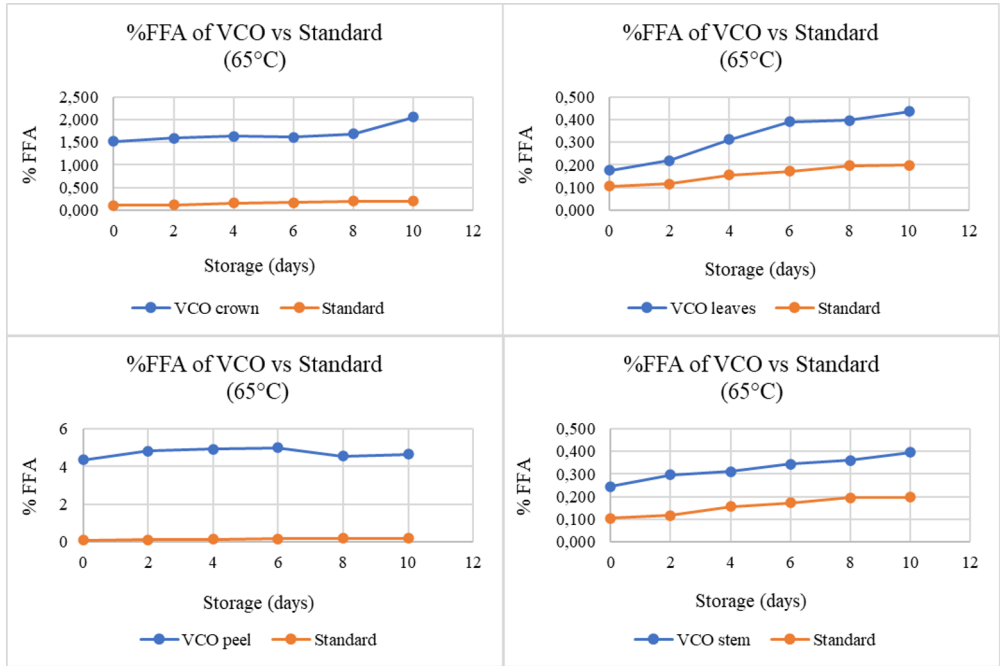


Fig. 4. Free fatty acid of VCO and standard at 65°C.

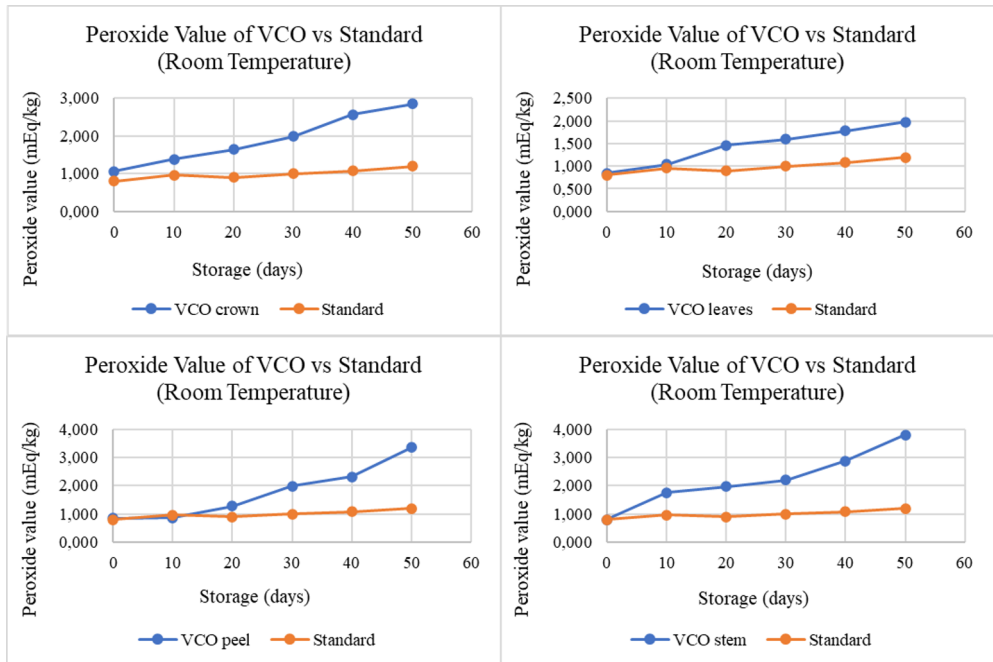


Fig. 5. Peroxide value of VCO and standard at room temperature.

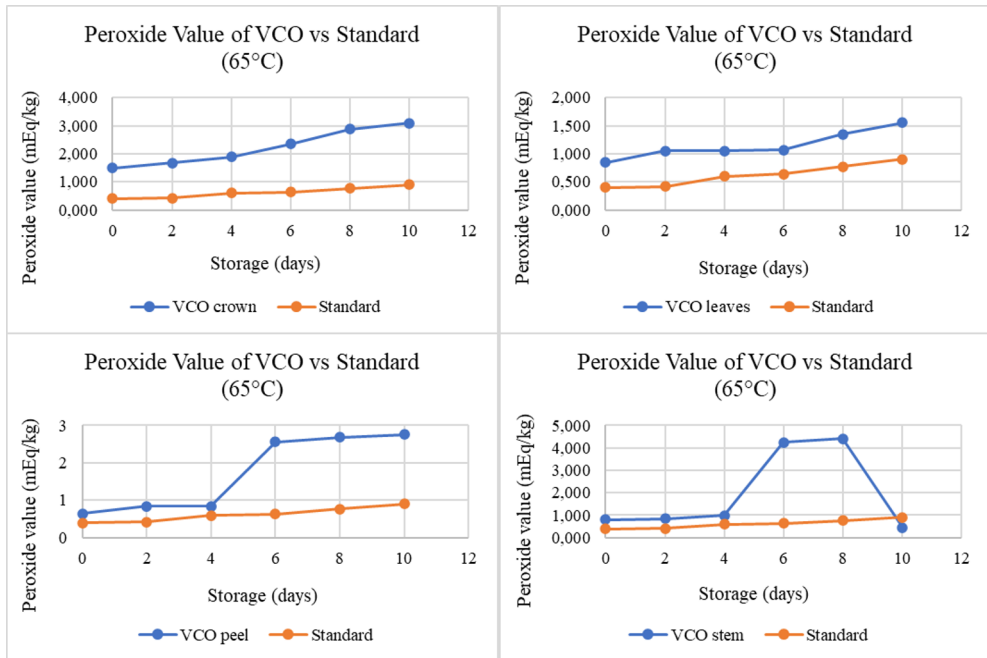


Fig. 6. Peroxide value of VCO and standard at 65°C.

The peroxide value test showed mixed results across VCO samples. This value measures total peroxides in oil, indicating rancidity and storage quality. Crown VCO met SNI standards (≤ 2 mEq/kg) at room temperature until day 30 but exceeded limits on days 40 and 50 (Fig. 5). At 65°C, it met standards until day 4 but exceeded them from day 6 onward (Fig. 6). Standard VCO stayed within limits at both temperatures. Peel and stem VCO showed similar patterns, meeting SNI at room temperature until day 30 and at 65°C until day 4. High temperatures accelerate oxidation in VCO, producing peroxides, aldehydes, ketones, and other short-chain fatty acids [17]. Leaf VCO samples showed different results, meeting SNI peroxide value requirements at both room temperature and 65°C (Figs. 5 and 6).

The final parameter, water content, affects shelf life, the lower is the better. Crown VCO samples exceeded the SNI limit ($>0.2\%$) (Figs. 7 and 8), likely due to added water during coconut milk preparation and the natural water content in older coconuts. Excess water accelerates hydrolysis, causing oil spoilage marked by off-odours and taste, reducing storage life. Pineapple leaf VCO showed poor moisture content at room temperature, meeting SNI only on days 20, 40, and 50 (Fig. 6). At 65°C, only day 0 failed, while days 2 onward met standards (Fig. 7). Pineapple peel VCO failed SNI moisture limits at both temperatures, likely due to its high water content (49.42–98.31%) (Figs. 6 and 7). Stem VCO samples showed varied results: moisture content exceeded SNI limits at room temperature (Fig. 6) but met standards at 65°C (Fig. 7)

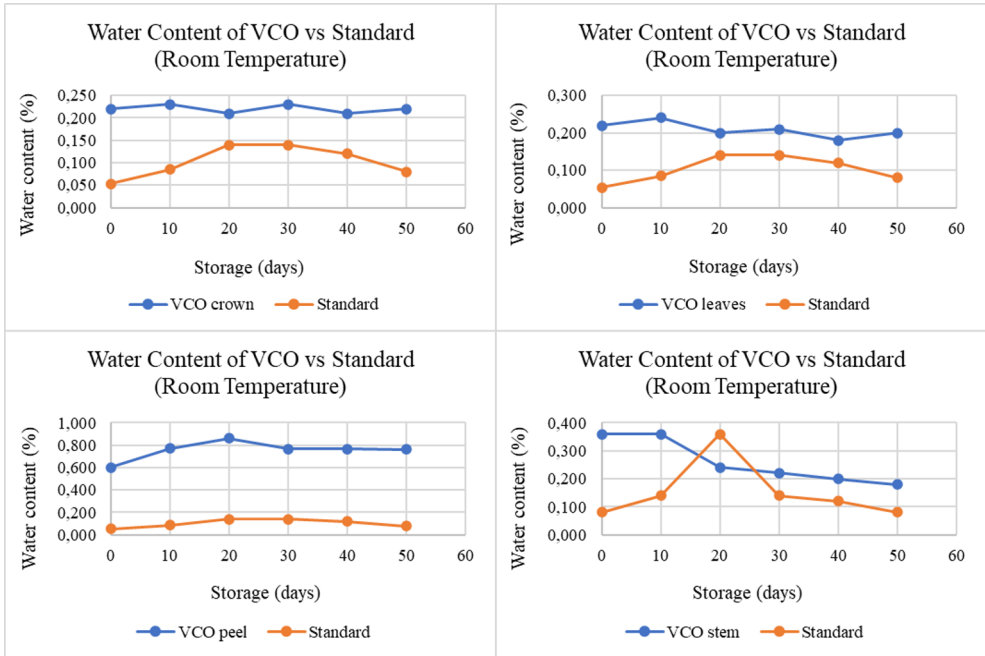


Fig. 7. Water content of VCO and standard at room temperature.

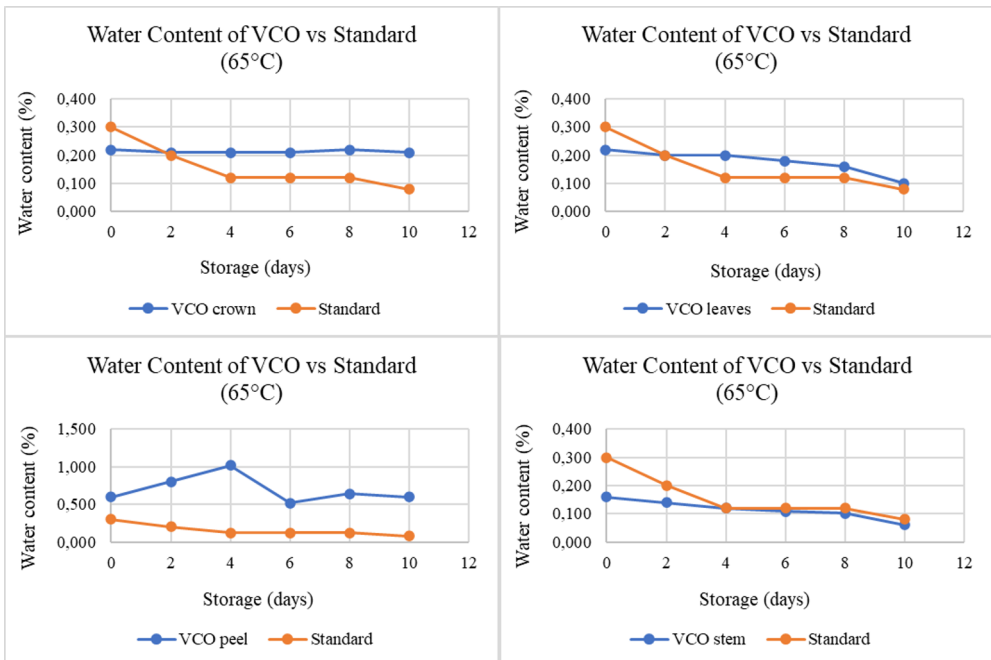


Fig. 8. Water content of VCO and standard at 65°C.

4 Conclusion

Temperature and storage duration impact the physicochemical stability of VCO from different pineapple plant parts. Crown VCO met SNI physical stability standards but failed chemical criteria like free fatty acid levels and moisture content. Its peroxide value stayed within limits until day 30 at room temperature and day 4 at 65°C. Leaf VCO maintained physical stability until day 40 (room temperature) and through the entire test at 65°C. However, free fatty acid and peroxide values increased over time at both temperatures, while moisture content fluctuated at room temperature and decreased at 65°C. Peel VCO met physical stability standards but failed for free fatty acid and moisture content. Stem VCO met physical stability requirements for colour and taste, though odour became rancid by days 40 and 50 at room temperature and from day 2 at 65°C. Free fatty acid and peroxide values rose at both temperatures, while moisture content decreased

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