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Lipid Quality Assessment of Virgin Coconut Oil Produced with Different Blanching Methods

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***Abstract*—Virgin Coconut Oil (VCO) is prone to oxidation due to the natural enzymes present in the coconut. Blanching has been studied to inactivate lipase and peroxidase enzymes that create deterioration in the product. Adding citric acid in hot water during blanching resulted in a higher quality product due to complexing agent properties to chelate metal substances and lowering the pH to reduce the enzymatic activities of the products. This research aims to analyze the effect of different blanching methods on VCO's lipid quality, antioxidant properties, and total phenolic content. Fresh coconut was subjected to water blanching at 95ºC for 5 minutes and 80ºC for 10 minutes with and without 0.05% citric acid addition. After that, VCO was produced using the chilling thawing method, which combines centrifugation and low-temperature incubation. VCO was analyzed in the free fatty acid (FFA), iodine value (IV), peroxide value (PV), TBARS value, the antioxidant activities by ABTS and DPPH, and total phenolic content (TPC). The results showed that water blanching with 0.05% citric acid at 80ºC for 10 minutes obtained better lipid quality compared to control with high IV (6.612±0.085 g iodine/g sample), low FFA (0.0241±0.0023 mg KOH/g sample), and low TBARS value (2.76±0.23 mg MDA/g sample). However, there is a contrary result between antioxidant activities and TPC that might reveal exciting findings. Despite a decrease in the antioxidant activities (DPPH: 39.93±3.11%RSA, ABTS: 9.80±0.91%RSA), the TPC value was increased (9.16±1.56 mg GAE/g sample). Therefore, hot water blanching with 0.05% citric acid addition can be conducted as a pre-treatment in the production of VCO to maintain the lipid quality, even though there is a contradiction in the TPC and antioxidant results.**

***Keywords*— VCO; blanching; chilling thawing; lipid quality; antioxidant.**

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I. INTRODUCTION

Virgin coconut oil, or VCO, is freshly extracted from a mature coconut kernel (*Cocos nucifera* L.) that can be extracted mechanically or naturally without altering and transforming the oil [1]. Coconut oil has beneficial health effects, reducing fat accumulation, improving lipid profile, increasing lipoprotein lipase, weight loss, antimicrobial agents, reducing CVD risk, lowering cholesterol level, and improving cognitive function [2], [3]. Medium-chain triglycerides (MCTs) contained in coconut oil are quickly absorbed and metabolized into energy, reducing the risk of high total cholesterol levels in the blood [4]. Biological active substances are also contained in the VCO, including sterols, phenolic compounds, and tocols [5]. Coconut oil has many functions, such as cosmetics, moisturizers, diarrhea and skin inflammation ailments, and anti-obesity treatment [1].

The chilling-thawing method is promising for creating VCO and retaining its nutritional value since it uses low-temperature processing. This method has several advantages, such as providing higher yield, less expensive, no chemical addition, and higher antioxidant qualities of the oil [6]. There are three processes to destabilize coconut milk, starting with centrifugation to separate the aqueous and cream layers. After that, the cream layer is frozen to conduct flocculation and clustering by destabilizing the emulsion and solidifying the oil. The last stage is when the interfacial regions are disturbed, leading to coalescence due to the thawing process that disturbs the spherical shape of the emulsion [7].

Deterioration of VCO can occur during processing and storage. During storage, the reduction of VCO quality and attributes can be caused by external factors such as light exposure, packaging material, and oxygen presence [8]. During extraction, the oil interacts with the remaining water, initiating hydrolysis, increasing free fatty acid, and creating unpleasant odors and flavors. Moreover, VCO deterioration can also be caused by hydrolytic rancidity by chemical or enzymatic hydrolysis by lipase and peroxidase naturally

present in coconut [9]. Hydrolysis also affects antioxidant availability by reducing the number of bioactive compounds in VCO, including phenols, unsaturated fatty acids, and tocopherols [8].

Blanching is a pre-treatment in the production process that can be done by applying heat to the raw material by immersing it in hot water or subjecting it to steam. This process can inactivate undesirable natural enzymes such as peroxidase, polyphenol oxidase, and lipoxygenase in several materials [10], [11], [12]. Blanching with citric acid can increase the bioactive compounds because of the chelating ability of citric acid as a metal complexing agent [13]. Moreover, citric acid reduces the plant tissue pH and suppresses the formation of enzymatic products that can trigger hydrolysis, such as radical compounds [14]. Inactivation of those enzymes is essential to prevent hydrolysis, oxidation, and browning. The other objectives of blanching are reducing the number of microbes, eliminating air pockets in the fruit and vegetable tissue, and reducing the firmness of the raw material [15].

There is a lack of studies about applying blanching in lipid preservation during VCO production, which is prone to deterioration. Correlating the lipid properties and antioxidant activities, as well as the total phenolic content of VCO with different blanching processes, might be an interesting topic to be explored further. Therefore, in this study, the different blanching methods were subjected to the coconut flesh, namely high- and low-temperature hot water blanching and hot water blanching with the addition of 0.05% citric acid. VCO was produced using the chilling thawing method to preserve the nutritional compound in the oil. Subsequently, the effect of the different blanching methods on the lipid quality of VCO was examined in this research by analyzing free fatty acid, iodine value, peroxide value, and TBARS value. Antioxidant activities were measured using DPPH and ABTS, and total phenolic content was also determined in this research.

II. Materials and Method

A. Materials

Coconuts were obtained from the local market in Jakarta, Indonesia, and had the same maturity and were assessed with the color of husk. Coconuts proceeded through a de-shelling and de-husking process, cleaned with water. Cutting processes were conducted, resulting in 16 triangular-shaped coconuts with similar dimensions. Samples were stored in the refrigerator before further processing. Reagents required for the analysis were methanol, glacial acetic acid, sodium carbonate, thiobarbituric acid, Folin reagent, 1-butanol, hexane, and tetraethoxypropane from Merck, Germany; DPPH from Himedia, India; gallic acid and ABTS from Sigma-Aldrich, USA; and potassium persulfate from Loba Chemie, India.

B. Blanching Method

This study performed two blanching methods: hot water blanching and hot water blanching with citric acid addition. Water blanching was conducted according to a study [16], and another blanching method was performed according to another study [17] by adding 0.05% citric acid to the hot water blanching. Different temperatures and blanching times were also carried out by high temperature at 95ºC for 5 min and low temperature at 80ºC for 10 min. High-temperature processing was combined with a shorter time to prevent excessive denaturation of nutritional value. In comparison, low temperature was combined with a longer time to ensure the inactivation of natural enzymes. The control sample was provided without blanching treatment. Samples were stored in the refrigerator before further processing. The sample was named according to the pre-treatment process applied, namely:

T1: control sample without blanching
T2: blanching at 95ºC for 5 minutes
T3: blanching with 0.05% citric acid at 95ºC for 5 minutes
T4: blanching at 80ºC for 10 minutes
T5: blanching with 0.05% citric acid at 80ºC for 10 minutes

C. VCO Extraction by Chilling Thawing Method

Coconut was shredded and pressed to obtain coconut milk. After that, extraction VCO using the chilling thawing method was performed, modified from a study [18]. Coconut milk was transferred into 50ml falcon tubes, and centrifugation was performed at 4000g for 12 min at room temperature to obtain two layers. The cream was collected and chilled until 5ºC. After that, the cream was stored in the refrigerator at 4ºC for 4 hours. Thawing was performed until the sample reached 25ºC. Then, the sample was centrifuged at 4000g for 20 min at 35ºC. The upper layer was collected using a micropipette and stored in a covered container in the refrigerator to be analyzed further.

D. Lipid Quality Analysis

1) Free Fatty Acid: FFA measurement was carried out to measure lipid hydrolysis, converting triglycerides to glycerol and free fatty acids. The measurement was done by using the method from AOCS Ca 5a-40, modified from previous work [19]. Ethanol in 50 mL was added by 2-3 drops of 1% phenolphthalein (PP) indicator and neutralized by titrating the solution with 0.1M NaOH until it became faint pink. VCO sample was added into the flask with 56.4±0.2 g, then added with neutralized alcohol. The sample was heated at 80ºC until dissolved with stirring. After that, 2-3 drops of 1% PP were dropped into the flask and titrated with 0.1 M NaOH until a faint pink color persisted for 30 s. FFA was expressed as lauric acid with the equation below:

$$Free\ fatty\ acid\ as\ lauric\ acid = \frac{mL\ of\ alkali\ x\ M\ x\ 20}{mass,g\ of\ sample} \quad (1)$$

The free fatty acid value can be defined as mg KOH to neutralize 1 g sample.

2) Iodine value: The iodine value was measured to indicate the lipid properties of the sample as it shows the unsaturation level of fatty acid. The iodine value measurement was performed using the AOCS Cd 1- 25 method, as conducted previously in a study [20]. The mixture of 0.1 g sample and 20 ml cyclohexane was produced before adding 25 ml Wijs solution. After incubation for 30 minutes in a dark place, 20 ml potassium iodide solution with 100 ml distilled water was incorporated into the mixture. The titration was done with 0.23 M sodium thiosulphate solution until it became light yellow before incorporating three drops of starch

solution. The titration was performed until the color disappeared. The blank sample was also prepared without any sample added to the mixture. The equation for calculating the iodine is shown below:

$$Iodine\ value = \frac{(B-S)\ x\ N\ x\ 12.69}{mass,g\ of\ sample} \quad (2)$$

where B = volume titration of the blank, S = volume titration of the VCO, and N = normality of sodium thiosulphate. The result was expressed as g iodine absorbed per g sample (% iodine absorbed).

3) Peroxide value: Peroxide value measurement was conducted using the AOCS Ca 8-53 method, modified from [21]. Sample (5g) was incorporated with 30 mL of 0.1 M acetic acid-chloroform solution and shaken for 1 min. After that, a saturated KI solution was added to 0.5ml and shaken gently. The sample was added with 30 mL of distilled water with intermittent shaking. After that, the sample was titrated with 0.01 N sodium thiosulfate until the yellowish color disappeared. The starch solution of 1% was added in 3 drops and continue the titration. A blank reading was recorded, and the peroxide value was calculated using the following equation:

$$Peroxide\ value = \frac{(volume Na_2SO_3 blank - volume Na_2SO_3 sample\ x\ N}{mass,g\ of\ sample}\ x\ 100 \quad (3)$$

The peroxide value was expressed as milliequivalents of peroxide per kilogram sample (meq peroxide/kg sample).

4) TBARS: TBARS assay was carried out to analyze the degree of lipid oxidation in the samples. The method used was modified from previous research [22]. The sample of 1 g was mixed with 5 mL water and vortexed for 2 min, continued by 3000 rpm centrifugation for 10 minutes. The aqueous layer was taken, and the method was repeated two times for malondialdehyde (MDA) extraction. Then, the extract of 2.5 mL was mixed with 2.5 mL TBA reagent containing 46 mM in 99% glacial acetic acid. The solution was heated for 35 minutes using a water bath and then chilled. The absorbance was measured using a spectrophotometer at a wavelength of 532 nm. A TEP (1,1,3,3-tetraethoxypropane) was used as a standard to compare the result expressed as mg MDA/g sample.

E. Antioxidant Activities and Phenolic Content Analysis

1) DPPH: The antioxidant activities were measured by DPPH assay, modified from a previous study [23]. VCO extraction was done by dissolving 10 g oil in 50 ml hexane, and the solution was extracted with 20 ml of 60% methanol, then evaporated using a rotary evaporator at 60ºC. After that, 10 mg extract was dissolved using 1 ml methanol. DPPH methanolic solution 0.8 mM was transferred to a test tube with the amount of 0.3 mL before the addition of 300µl VCO extract. After being shaken vigorously, incubation was performed in a dark place at room temperature for 30 min, and then the absorbance was measured using a spectrophotometer at 517nm of wavelength. The percentage of DPPH radical scavenging activity was calculated with the equation below:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ sample}\ x\ 100 \quad (4)$$

where Abs control is the absorbance of blank solution as a control, and Abs sample is the absorbance of the VCO solution.

2) ABTS: The ABTS method, modified from the previous study, was used to measure the antioxidant activities of VCO [24]. VCO extraction was performed by diluting 10 g VCO in 10 mL hexane, and the solution was extracted with 20 mL methanol: water (60:40, v/v) 3 times. After shaking, 0.4 mL sample solution was diluted with 5mL 1-butanol until 4% (v/v) hydrophilic extract solution was obtained. The sample extract (0.5 mL) was mixed with 2 mL of the ABTS reagent and then incubated at room temperature for 15 minutes in dark conditions. The absorbance was measured with spectrophotometry at a wavelength of 734 nm. The percentage of ABTS radical scavenging activity was calculated with the equation below:

$$ABTS\ radical\ scavenging\ activity\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ sample}\ x\ 100 \quad (5)$$

where Abs control is the absorbance of blank solution as a control, and Abs sample is the absorbance of the VCO solution.

3) Total Phenolic Content: Phenolic content was measured using TPC and examined using the 7 Folin-Ciocalteu technique, as mentioned by [25]. Multiple concentrations of gallic acid were used for the standard curve. The TPC analysis of the samples was started by extracting polyphenol compounds in the VCO. VCO of 10 g in 50 mL hexane was extracted with 80% methanol thrice. The extract was evaporated using a rotary evaporator at 40ºC [26]. The extract samples of 0.3mL were mixed with 1.5 mL of 10 times diluted Folin reagent in the test tube. After that, 1.3 mL of 7.5% sodium carbonate in a water solution was added and mixed using a vortex. After incubation in a dark place for 30 minutes at room temperature, the absorbance was measured at 760 nm with spectrophotometry. The total phenolic content was expressed as mg GAE/ gram VCO sample.

F. Statistical Analysis

After data collection, statistical analysis was conducted using SPSS statistical software ver. 25.0. Analysis of variance (ANOVA) was used to compare the mean differences between the samples, followed by a multiple comparisons test using Tukey HSD with a significant level of 5%.

III. Results and Discussion

A. Lipid Quality

The lipid quality of VCO was analyzed with the measurement of free fatty acid (FFA), iodine value (IV), peroxide value (PV), and thiobarbituric acid reactive substances (TBARS). The result of the lipid quality analysis is presented in Table 1.

1) Free Fatty Acid: The measurement of FFA was carried out to determine the level of hydrolysis of triglyceride in the VCO sample. The result of the FFA value of the VCO sample with different pre-treatments can be seen in Table 1.

TABLE I
LIPID QUALITY ANALYSIS RESULT OF VCO WITH PRE-TREATMENT PROCESS

Sample	Free Fatty Acid (mg KOH/g sample)	Iodine Value (g iodine/g sample)	Peroxide Value (meq peroxide/ kg sample)	TBARS Value(mg MDA/g sample)
T1: Control	0.0320±0.0009[a]	5.877±0.079[a]	0.0106±0.0083[a]	5.62±0.12[a]
T2: 95ºC, 5 min	0.0306±0.0021[ab]	6.003±0.082[ab]	0.0259±0.0086[a]	5.76±1.27[a]
T3: 95ºC, 5 min + citric acid	0.0206±0.0012[c]	6.340±0.094[bc]	0.0159±0.0041[a]	5.40±0.59[a]
T4: 80ºC, 10 min	0.0300±0.0025[ab]	6.411±0.094[c]	0.0197±0.0054[a]	3.35±0.66[b]
T5: 80ºC, 10 min + citric acid	0.0241±0.0023[bc]	6.612±0.085[c]	0.0239±0.0113[a]	2.76±0.23[b]

Blanching with citric acid showed the different FFA values between the VCO samples. FFA value was decreased with the pre-treatment of blanching and the addition of citric acid during blanching. Moreover, a lower FFA value was obtained in the blanching at 80ºC for 10 minutes. The results indicate that blanching inhibits lipid hydrolysis during storage. Based on [15], the FFA of the VCO with blanching is lower than the control due to the inactivation of lipase that can hydrolyze oil during processing. Moreover, according to [27], the FFA of super-heated steam-treated buckwheat grain was lower than untreated after storage due to the inactivation of lipase enzyme, causing the low release of free fatty acid. In addition, citric acid has chelating ability properties, acting as a metal complexing agent and preventing metal from oxidizing lipids [13]. Moreover, the low pH of citric acid reduces the reaction rate of radical compound production [14]. This result did not align with the FFA result, which showed decreased antioxidants due to blanching. The reduction might be the reason for partially washing out the initial FFA in the coconut flesh during blanching [16]. Blanching with citric acid decreases the formation of free fatty acid in VCO.

2) Iodine value: The determination of iodine value was conducted to measure the susceptibility of VCO toward lipid oxidation. Table 1 shows the result of iodine value measurement on the VCO with different blanching methods. After the blanching process, the iodine value increased at a lower temperature and with the addition of citric acid during blanching. The iodine value of all samples showed a low value compared to the Asian and Pacific Coconut Community, which is around 4-11 g I_2/100 g fats due to the saturation degree of VCO. The precision might cause a slightly different value from samples during titration [1]. A low iodine value indicates that VCO is resistant to rancidity due to better oxidative stability. Moreover, IV correlates with overall quality properties such as sensory properties and shelf life [28].

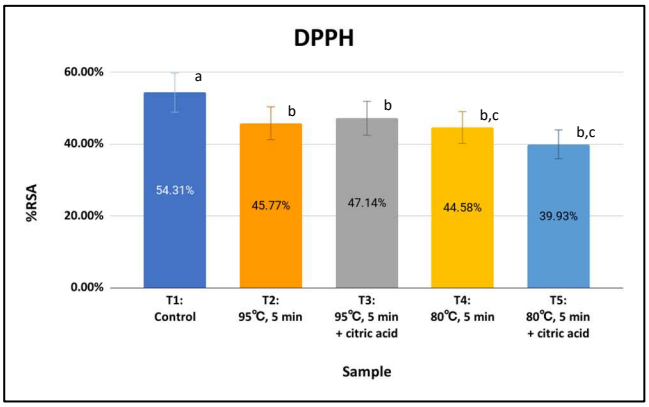
3) Peroxide value: Peroxide value measurement was done to assess peroxide production due to oxidation. The result of the peroxide value of different VCO samples is shown in Table 1. The peroxide value of the VCO with blanching was similar to that of the control without blanching. The results indicate no oxidation by the peroxidase enzyme during the process because VCO contains no protein. According to [16], blanching of coconut kernel results in low peroxide value and reduction in the activities of peroxidase enzyme as well as lipoxygenase enzyme, proofing that the inactivation of peroxidase and lipoxygenase enzyme causes the decrease of peroxide value. Blanching creates lower peroxide value because blanching prevents oxidation by lipoxygenase and peroxidase that produce peroxide [15]. The result was contrary to the previous study due to different extraction processes. VCO extraction using the chilling thawing method was done at low temperatures without a drying process, resulting in no susceptibility towards oxidation by peroxidase.

4) TBARS: TBARS measurement was done to determine lipid oxidation by calculating (malondialdehyde) MDA concentration in the sample. The result of the TBARS value is presented in Table 1. Blanching resulted in different TBARS values between the VCO sample, especially blanching with the low temperature at 80ºC for 10 minutes (T4 and T5). This result was linear with the antioxidant activities result, and the antioxidant activities were decreased because of the prevention of the oxidation process, resulting in a low MDA value. According to [29], blanching at 80ºC for 20 min inhibits peroxidase formation compared to unblanched cane stalk during sugarcane juice production. Furthermore, the similar peroxide value might indicate that the primary oxidation was started, but antioxidant compounds can prevent the secondary oxidation. Therefore, the MDA value can be reduced in the sample with lower antioxidant activities.

B. Antioxidant Activities and Phenolic Content

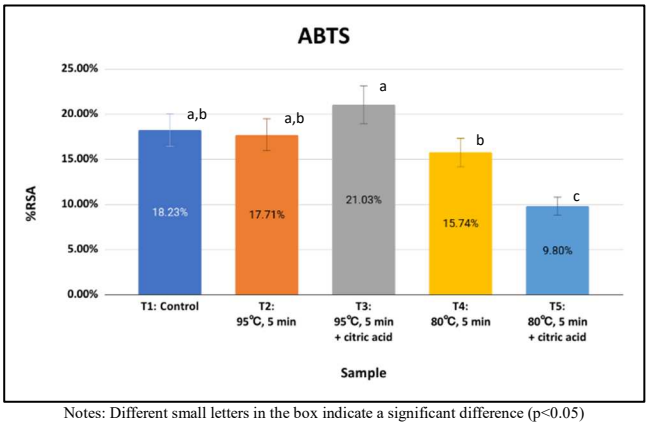
1) Antioxidant activities: Antioxidant activities were carried out using DPPH and ABTS methods, with the results shown in Fig 1 and Fig 2. DPPH radical scavenging activities showed that the blanching method reduced the antioxidant activities compared to the sample without blanching (T1). The highest decrease was the blanching with citric acid at 80ºC for 10 minutes (T5), while other blanching methods resulted in a similar result. The antioxidant activities were decreased in the T2 sample, followed by T3, T4, and T5. The result of antioxidant activities by the ABTS method obtained similar results in VCO with pre-treatment except for T5. The antioxidant activity was identical between T1, T2, T3, and T4 but decreased in T5. The blanching process with citric acid 0.05% at 80ºC for 10 minutes created lower antioxidant activities in the VCO sample than without pre-treatment. The result between DPPH and ABTS was linear. However, the slight difference in the percentage of RSA might be due to the sensitivity of each measurement. According to [30], heating at a high temperature decreases the antioxidant activities of some products due to protection from antioxidant compounds against thermal oxidative degradation. Based on the previous study [31], phenolic compounds and medium-chain fatty acids promote antioxidant activities in virgin coconut oil as bioactive compounds, scavenging free radicals, chelating metal ions, and preventing lipid oxidation. The different types

of bioactive compounds in the VCO create interesting results between antioxidant activities and total phenolic content results, which show a contrary result.



Notes: Different small letters in the box indicate a significant difference ($p<0.05$)

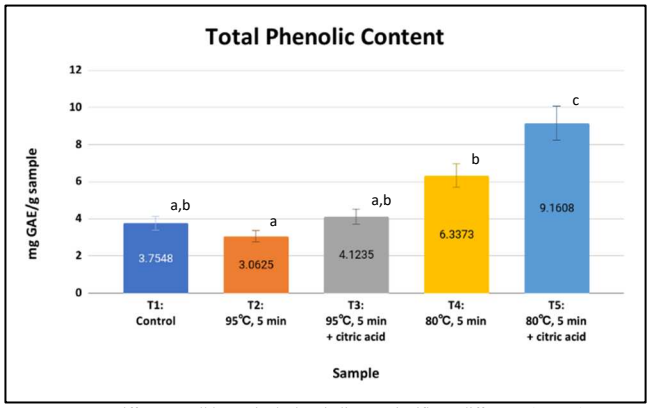
Fig. 1 DPPH of virgin coconut oil with different pre-treatment processes



Notes: Different small letters in the box indicate a significant difference ($p<0.05$)

Fig. 2 ABTS of virgin coconut oil with different pre-treatment processes

Total Phenolic Content: Total phenolic content was measured in this work to determine the level of bioactive compounds after pre-treatment. The result of the total phenolic content on the VCO can be seen in Fig 3. Sample with pre-treatment blanching increased the phenol content in the VCO, followed by T3. T4, and T5. The phenolic content of the VCO with the blanching added by citric acid at 80ºC for 10 minutes (T5) obtained the highest value. However, blanching with a high temperature of 95ºC for 5 minutes (T2) showed a similar result in the total phenol content compared to the sample without pre-treatment. The result was contrary to the result of antioxidant activities, which showed a decrease in the sample with the blanching process. This might be caused by other bioactive compounds rather than phenol, which play a role in the antioxidative properties of VCO. Other than phenolic compounds, medium-chain fatty acids are available in the VCO that might degrade during blanching, resulting in lower antioxidant activities [31]. Based on [32], phenolic compounds are mostly hydrophilic with many hydroxyl groups and more soluble in water; the compounds were removed during VCO extraction. On the other hand, phytosterols, another source of bioactive compounds, are hydrophobic and are concentrated in the VCO in approximately 90mg/100g VCO. The higher amount of phytosterols showed that phytosterols play a more important role in the antioxidant activities of VCO than polyphenols. Phytosterol might degrade during the blanching process, resulting in lower antioxidant activities. Therefore, the amount of polyphenol was still high even though the antioxidant activities result showed a lower value in the sample with the blanching process.



Notes: Different small letters in the box indicate a significant difference ($p<0.05$)

Fig. 3 Total phenolic content of virgin coconut oil with different pre-treatment processes

IV. Conclusion

Blanching as a pre-treatment in the coconut kernel affects the lipid quality of VCO extracted using the chilling thawing. Water blanching at lower temperatures and more extended time with the addition of 0.05% citric acid resulted in the best lipid quality of VCO. Water blanching with 0.05% citric acid at 80ºC for 10 minutes showed high IV (6.612±0.085 g iodine/g sample), low FFA (0.0241±0.0023 mg KOH/g sample), and TBARS value (2.76±0.23 mg MDA/g sample). However, contrary to previous studies, the antioxidant activities were decreased. Still, higher TPC was obtained (9.16±1.56 mg GAE/g sample), showing different bioactive compounds such as phytosterols and medium-chain fatty acids might play a role in promoting the antioxidant activities. These findings might give VCO producers and consumers new insight regarding the blanching process that can prevent lipid deterioration, such as rancidity and hydrolysis, resulting in higher-quality VCO products.

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