

Virgin Coconut Oil (VCO) Decreases the Level of Malondialdehyde (MDA) in the Cardiac Tissue of Experimental Sprague-Dawley Rats Fed with Heated Palm Oil

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Abstract—Heating of edible oils maybe harmful for human consumption. However, with the advent of newer oils like virgin coconut oil, it is interesting to observe the effects of the oil on inflammatory markers associated with cardiovascular diseases. The present study aimed to investigate the influence of virgin coconut oil on the malondialdehyde level in the heart tissue of rats fed with heated palm oil. Thirty two male Sprague-Dawley rats (200-280 g) were equally assigned into four groups and fed as follows: Control-group with normal rat chow; VCO-group with rat chow and supplemented with 1.43ml/kg body weight of VCO; Five times heated palm oil (5HPO)-group with rat chow fortified with 15% weight/weight (w/w) of 5HPO; and 5HPO + VCO-group with rat chow fortified with 15% w/w of 5HPO plus 1.43ml/kg body weight of VCO simultaneously. The treatment duration continued for four months. Thereafter, the thirty two rats were sacrificed and heart tissues were harvested for biochemical analyses. There was a significant ($p < 0.05$) decrease in peroxide value in the VCO. The MDA level in the VCO and 5HPO+VCO groups was reduced significantly ($p < 0.05$) compared to the 5HPO group. In conclusion, VCO supplementation reduced the oxidative stress as depicted with decrease in peroxide value and MDA level.

Index Terms—virgin coconut oil, peroxide, inflammatory, heated palm oil, malondialdehyde, cardiac tissue

I. INTRODUCTION

Palm oil is obtained from the tropical plant and its scientific name is *Elaeis guineensis*. Palm oil has saturated - unsaturated fatty acid ratio close to one and naturally very stable due to its chemical properties. The practices of using reheated oil and deep frying are very common in food preparation process and palm oil is the most common vegetable oil used [1]. However, the heating process causes changes in palm oil composition and its healthy properties. Repeatedly heated oil undergoes changes in physical appearance and a series of chemical reactions such as oxidation, hydrolysis and

polymerization that eventually alter the fatty acid composition [2]. Therefore, when the degree of unsaturation in fatty acid is greater, it is more vulnerable to lipid peroxidation [2].

Malondialdehyde (MDA) is one of the major end products of lipid peroxidation which causes endothelial damage, vascular inflammation and cell membrane injury [3]. Oxidative degradation of heated palm oil was proven to produce higher peroxide values [4]-[6] and increased in plasma MDA level [6].

Lately, the properties of virgin coconut oil (VCO) have been broadly investigated due to its antioxidant actions. VCO is extracted directly from coconut (*Cocos Nucifera* Linn) milk by a wet process under controlled temperature thus it has more beneficial effects than copra oil (CO) since VCO could retain most of its unsaponifiable components. It contains fatty acids which are mainly saturated fatty acid (medium chain fatty acid) and unsaturated fatty acid as minority. Thus, a study report [7] showed that the peroxide value of VCO was even lower than the fresh palm oil and this was suggested as a result of its increased oxidative stability. Obtaining the peroxide value of oil would provide us the value of its oxidation and perhaps to educate us on the safety of the concerned edible oil. In addition to this finding, they also found that the high phenolic acid content plays its actions as well to reduce the oxidation process [8]. Subsequently, another study [9] showed that polyphenol fraction in VCO could reduce lipid and LDL oxidation significantly.

Consumption of VCO was found to completely abolish the expected immune factor responses to endotoxin and diminishes the production of pro-inflammatory cytokines in vivo. In earlier studies, it was revealed that virgin coconut oil exhibited its therapeutic values of anti-inflammatory, anti-thrombotic [10]; in experimental rats and contains antioxidant properties [11]. Oxidation of low density lipid was prevented by the phenolic fraction which was separated from VCO and in addition to that, antioxidant status was increased among the rats fed with VCO supplemented diet [9], [11].

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A number of research findings had explained the adverse effects on human and experimental animals by the oxidized fat in diet. More specifically, the effects of heated palm oil on cardiovascular diseases have been proven by many local and international researchers. The main aim of this study was to determine the protective effect of virgin coconut oil on the peroxide value of the fresh and heated oils and MDA level in the cardiac tissue.

II. MATERIALS AND METHODS

A. Palm Oil

The palm oil was purchased from local market (Cap Buruh, Lam Soon Edible Oil, Kuala Lumpur). It was used as fresh and heated five times, according to the modified method of Owu et al. [12].

B. Preparation of Heated Palm Oil Diet and Administration

The heating process of palm oil needed used 2.5 litres of oil to fry 1 kg of sweet potatoes bought from local market. The skin of the sweet potatoes was peeled and the sweet potatoes were cut into slices, thereafter fried in a stainless-steel wok at about 180°C for 10 minutes. After frying for 10 minutes, the sweet potatoes were removed from the oil and the heated oil was cooled down for five hours. Then the entire frying process was repeated with a fresh new batch of 1 kg sweet potatoes in the same oil without adding on fresh oil into it. This frying process was repeated for four times in order to get the five times heated oil. Standard rat chow (Gold Coin, Kepong, Malaysia) was ground and fortified by adding with 15% weight/weight (w/w) of the five times heated palm oil that had been prepared. The fortified rat chow was then reformed into pellets and dried in an oven at 80 °C overnight. This diet was stored in a closed cabinet and prepared weekly.

C. Virgin Coconut Oil and Administration

The virgin coconut oil was purchased from local manufacturer (Organic Gain Sdn Bhd., Termerloh, Pahang, Malaysia). Virgin coconut oil was fed fresh by means of oral gavage to the rats with the dosage of 1.43 ml/kg of body weight/day [13].

D. Animals

A total of thirty two (n=32) adult male *Sprague-Dawley* rats aged three months old (weighing 200-280g) were used for this study. These rats were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur with prior ethical clearance from Universiti Kebangsaan Malaysia Animal Ethical Committee (FP/ANAT/2012/FAIZAH/26-SEPTEMBER/457-SEPTEMBER-2012-AUGUST-2014). The handling and care perhaps all management of animals were conducted according to the recommended guidelines and experimental protocols approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). The experimental animals were housed individually in polyethylene with stainless-steel covered

cages. They were kept at room temperature of 27 °C ± 2 °C with 12-hours light-dark cycle in the Anatomy Department Animal House. All the experimental animals had free access (*ad libitum*) to food and tap water throughout the experiment period of four months. All the experimental animals were acclimatized for one week prior to the administration of the test diet and supplements.

E. Study Design

Thirty two (n=32) male *Sprague-Dawley* rats were equally assigned into four groups that comprised of eight rats each and they were given the following course of diet and supplement: (i) normal rat chow (basal diet) as control group, (ii) normal rat chow (basal diet) and VCO (1.43ml/kg/day body weight) supplement as VCO group, (iii) 15% w/w of five times heated palm oil with rat chow as 5HPO group and (iv) 15% w/w of five times heated palm oil with rat chow fortified with VCO (1.43ml/kg/day body weight) supplement as 5HPO+VCO group. After four months of experiment, animals were sacrificed and heart tissues were harvested and immediately stored and frozen at -80°C.

F. Peroxide Value

The peroxide content of palm oil and virgin coconut oil was measured using standard titration method (Official method Cd 8-53) according to the American Oil Chemists' Society (AOCS). This method required five grams of the oil sample that was added to 30 ml of acetic acid-chloroform (3:2) in a conical flask. Thereafter, the solution was swirled for 1 minute before and after adding 0.5 ml of saturated potassium iodide into the solution. Distilled water of 30 ml and few drops of starch solution (10%) were added and the solution was titrated against 0.01 N sodium thiosulphate solutions which were standardized by using potassium dichromate and potassium iodide. The titration was continued until the blue colour disappeared. The peroxide level was calculated as the difference in volume of sodium thiosulphate solution (ml) used for samples and blank, divided by its normality. The value of peroxidation was expressed as miliequivalents of active oxygen per kilogram (mEq O₂/kg) of the oil sample.

G. MDA Level

Lipid peroxidation in the samples' heart tissue homogenates, as determined by the MDA level was measured using Cayman's Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). Heart tissues were individually weighed and homogenized in 250 µl of RIPA Buffer Concentrate and protease inhibitors, using an automated homogenizer (OMNI Bead Ruptor-24, USA). The homogenates were then centrifuged at 3821 x 1000 rpm for 10 minutes at 4°C. Clear supernatants were used for the MDA assay. MDA concentration was measured colorimetrically at 530-540 nm on Emax ELISA microplate reader using Soft-Max Pro Software (Molecular Devices, Sunnyvale, CA, USA).

The protein content in the heart tissue was determined prior to TBARS assay by using a method which was described by Lowry et al [14]. A value of 0.5ml tissue supernatant was added with 5ml mixture of 2% natrium carbonate (Na_2CO_3), 2% kalium natrium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) and 1% copper sulphate solution ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) with the ratio of 100:1:1. Upon that, the solution was then left to stand at room temperature for 15 minutes before a volume of 0.5ml of diluted Folin-Ciocalteu phenol reagent was added to. Subsequently the solution was left in the room temperature for 35 minutes and the absorbency of the sample was measured at 700nm with spectrophotometer (Shimadzu UV-160A, Japan). The result of MDA level in tissue homogenate was measured as TBARS and was expressed as $\mu\text{M}/\mu\text{g}$ of protein.

H. Statistical Analysis

All the results were expressed as mean \pm SEM. Normality test of data was determined using Shapiro-Wilk test. The mean comparisons among groups were analysed using one-way analysis of variances (ANOVA) followed by Games-Howell post-hoc test. A value of $P < 0.05$ was considered as statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA).

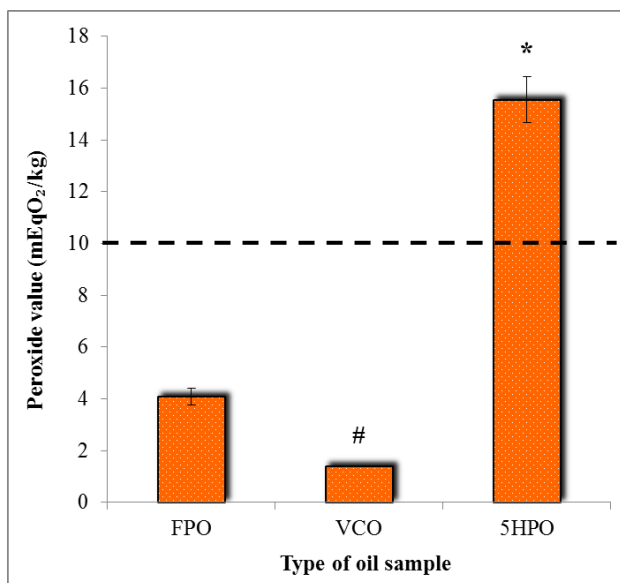


Figure 1. Peroxide value determined in three different oil samples: fresh palm oil (FPO), virgin coconut oil (VCO) and five times heated palm oil (5HPO). The bars represent mean and error bars, SEM with $n = 3$ in each group. * Significant difference ($p < 0.05$) were observed in 5HPO compared to FPO and VCO; # Significant difference ($p < 0.05$) in VCO compared to FPO. Dashed horizontal line indicates maximum allowable peroxide value for edible oils according to the American Oil Chemists' Society (AOCS).

III. RESULTS

The peroxide values showed a three-fold increment ($p < 0.05$) for FPO, and an eleven-fold increment for 5HPO, compared to the VCO value. There was a significant difference ($p < 0.05$) in peroxide level among the FPO, 5HPO and VCO samples (Fig. 1).

The 5HPO group showed significant increment ($p < 0.05$) in the heart tissue MDA level at the end of the study (Fig. 2). The MDA level showed significant difference ($p < 0.05$) between control and VCO, 5HPO, 5HPO+VCO; between 5HPO and VCO, 5HPO+VCO groups (Fig. 2).

IV. DISCUSSION

Peroxide level was obtained to determine the extent of oil degradation during cooking or heating. Hence, it measured the value of peroxides produced in the cooking oil during the process of oxidation as a result of heating.

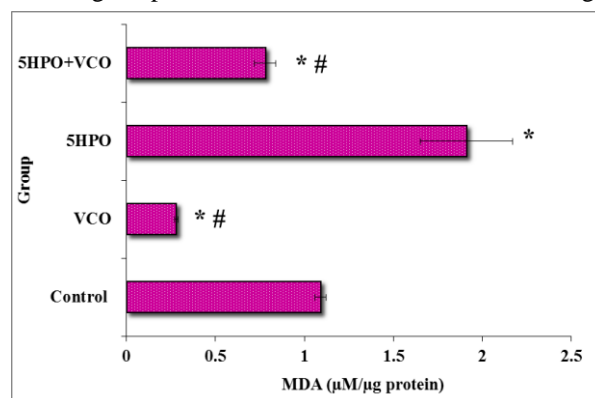


Figure 2. DA level in heart tissue after 4 months of feeding with basal diet (control), five times heated palm oil (5HPO), basal diet and VCO supplementation (VCO) and five times heated palm oil with VCO supplementation (5HPO+VCO). The bars represent mean and error bars, SEM, with $n = 8$ in each group. * Significant differences ($p < 0.05$) were observed in 5HPO, VCO, 5HPO+VCO compared to control group and * significant differences ($p < 0.05$) also been observed in VCO & 5HPO+VCO compared to 5HPO group.

Our results suggest that the extent of oxidation rancidity was very much influenced by the number of times the oil was heated. The more number of times the oil was heated, the higher was the peroxide level in the oil samples. Nevertheless, this was incongruent with previous study [6], who found the peroxide value of the heated palm oil was higher compared to fresh palm oil and beside that, five times heated palm oil showed very high peroxide value compared to once or twice heated oil. Thus it was suggested that palm oil is best used in fresh or heated once state. In our present study, we found that five times heated palm oil had greater peroxide value compared to fresh palm oil and VCO. As the peroxide value is being used as an indicator for oxidative stability and the extent of oil degradation, thus our result suggests that five times heated palm oil is not recommended for cooking perhaps should not be consumed. Peroxide value for 5HPO was noted to be beyond the upper limit of peroxide value set by the American Oil Chemists' Society (AOCS) which is 10 mEq O₂/kg oil [7].

Since the peroxide value is higher in the reheated palm oil, it indicates that its chemical stability upon oxidation is lower. This could be linked to the saturated fatty acid content in the palm oil which is only about 50% as compared to the VCO that has almost 90% of saturated fatty acid [3]. It is believed that heating at high temperature and furthermore repeatedly, has negative

impact on the fatty acid composition. Nevertheless, the presence of unsaturated bonds in the fatty acid chains gets attacked by the free radicals that are produced during the heating process [2], [8]. Though it is known that palm oil is rich in antioxidant vitamin E, namely abundant amount of tocotrienol and an amount of α -tocopherol that help to reduce the lipid peroxidation, but it was reported to have reduced of those vitamins during repeated heating process thus failed to maintain its oxidative stability [7].

In the present study, VCO showed the lowest peroxide value (below 1.5 mEq O₂/kg oil), which was even lower than fresh palm oil. However, to assess the extent of oxidation and chemical stability of an oil, peroxide value alone is not enough as such peroxides and hydroperoxides that had been generated during the heating process are unstable and could be decomposed easily to other compounds which ultimately could bring the peroxide index further down.

VCO which mainly has the medium chain fatty acid as its saturated fatty acid, is suggested to possess better oxidative stability with low peroxide value [8]. Though in our present study, peroxide value was only obtained in fresh VCO and not in the heated oil as per use in our study, we expect the oil to have lower peroxide value because of its phenolic acid content which has been reported to increase its scavenging activity towards free radicals, thus contributing to its antioxidant property [7], [10]. The results of the present study showed that the peroxide value of VCO was significantly lower compared to FPO and 5HPO. Our results are in accordance with earlier findings [8], [9] while the polyphenol fraction in virgin coconut oil could reduce lipid and LDL oxidation significantly [12]. However, we suggest that another study should be conducted to confirm the VCO's capability of oxidative stability upon heating and repeatedly heating.

Malondialdehyde (MDA) is a major end product of lipid peroxidation especially the polyunsaturated fatty acid peroxidation. Lipid peroxidation is used to monitor the oxidative stress in cells and tissues and it is a well-developed way of describing cellular injury [6], [11]. In the present study, MDA level in heart tissue of the control, VCO and 5HPO+VCO groups were significantly lower compared to 5HPO group. This suggests that consumption of repeatedly heated oil raised the lipid peroxidation in heart tissues. Besides that, MDA level of the heart tissues of the VCO group was found to be lower compared to the control group. This could be due to the phenolic fraction in VCO which had been reported [7], [9] to play a main role in scavenging activity of free radicals thus increases its antioxidant capability. Nevertheless, in the present study it was also observed that the MDA level in heart tissue of 5HPO+VCO group was significantly lower compared to 5HPO alone group. This result indicates that VCO has shown its protective effect by reducing the MDA level in the heart tissue which was affected by lipid peroxidation. Therefore, this finding suggests that VCO has the capability of reducing oxidative stress in cardiac tissue.

In the present study, the changes observed with regard to peroxide level of 5HPO could be related to the significant increase in the MDA level in the cardiac tissue of the 5HPO group and reduced MDA level in the VCO group with lowest peroxide value. This relation indicates that the repeated heating of palm oil increases its oxidation which eventually elevates the lipid peroxidation while VCO has a great oxidative stability as compared to palm oil.

V. CONCLUSION

In conclusion, VCO supplementation has the capability of reducing the oxidative stress and good effect on health due to its stability against oxidative insult which leads to cardiovascular diseases.

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