Effect of crude palm oil consumption on the levels of plasma ß-carotene, malondialdehyde and xanthine oxidase activity of healthy housewives

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ABSTRACT

Crude palm oil that contains high ß-carotene can be obtained from mesocarp extraction of *Elaeis guneensis* Jacq. In the body, the ß-carotene is converted to vitamin A that has antioxidant activity. Antioxidants can inhibit lipid peroxidation induced by free radicals or singlet oxygen. It also inhibits the increase of reactive oxygen species (ROS) in the form of anion superoxide and hydrogen peroxide through the xanthine oxidase mechanism. This study aimed to evaluate the effect of crude palm oil consumption on the plasma ß-carotene and malondialdehyde (MDA) levels as well as xanthine oxidase activity of healthy housewives. Twenty-two healthy housewives who met the inclusion and exclusion criteria were involved in this study. Blood plasma samples were taken before and after consuming crude palm oil for two months as much as ± 3.58 mL daily. The results showed that consumption of crude palm oil increased the plasma ß-carotene levels of 16 people (72%), and decreased the plasma MDA levels of 13 people (59%). Meanwhile, the xanthine oxidase enzyme activity showed no significantly different (p>0.05). Crude palm oil can increase plasma ß-carotene levels. It can be an alternative natural food source of provitamin A due to the high ß-carotene content.

ABSTRAK

Minyak sawit yang mengandung ß-karoten tinggi dapat diperoleh dari ekstraksi mesokarp tanaman *Elaeis guneensis* Jacq. Dalam tubuh ß-karoten dikonversi menjadi vitamin A yang mempunyai aktivitas antioksidan. Antioksidan dapat menghambat peroksidasi lipid yang diinduksi radikal bebas atau oksigen singlet. Antioksidan juga menghambat kenaikan reactive oxygen species (ROS) berupa anion superoksida dan hydrogen peroksida melalui mekanisme ksantin oksidase. Penelitian ini bertujuan untuk mengkaji efek konsumsi minyak sawit mentah terhadap kadar ß-karoten plasma darah, malondialdehid (MDA) dan aktivitas ksantin oksidase. Duapuluh dua ibu rumah tangga sehat yang memenuhi kriteria inklusi dan eksklusi dilibatkan dalam penelitian ini. Sampel plasma darah diambil sebelum dan sesudah mengkonsumsi minyak sawit mentah selama 2 bulan sebanyak ± 3.58 mL setiap hari. Hasil penelitian menunjukkan bahwa konsumsi minyak sawit mentah meningkatkan kadar ß-karoten plasma pada 16 orang (72%), serta menurunkan kadar MDA pada 13 orang (59%). Namun demikian, aktivitas enzim ksantin oksidase tidak menunjukkan perbedaan bermakna (p>0.05). Minyak sawit mentah dapat meningkatkan kadar ß-karoten dan dapat dijadikan alternatif bahan pangan alami sumber provitamin A karena kandungan ß-karotennya yang tinggi.
INTRODUCTION

Crude palm oil can be obtained from mesocarp extraction of *Elaeis guineensis* Jacq plant. Unfractionated crude palm oil contains 514.7 mg/kg of ß-carotene and tocopherol component 800-1000 ppm. Palm oil production continues to increase every year, with total production of 31.49 million tons in 2016, while in 2017 it was estimated to have increased by 9.46 percent to 34.47 million tons. Generally, palm oil derivative products are cooking oil. In the process of making cooking oil, ß-carotene will be destroyed by decolorization (bleaching), so that a clear yellow cooking oil is obtained, with a carotene content that is much smaller than the initial content of the material. In Indonesia, the utilization of ß-carotene contained in palm oil has not been carried out optimally, because raw materials are only used for the manufacture of cooking oil. On the other hand, Malaysia already has red palm oil products that are mass produced to meet the needs of vitamin A in the community. Research on the use of crude palm oil has been widely carried out.

Some research reported that consumption of red palm oil can reduce endogenous cholesterol levels. This is due to the content of tocotrienols as well as a distinctive position isomers of fatty acids. Red palm oil is beneficial for reducing the risk of arterial thrombosis/atherosclerosis, inhibiting the biosynthesis of cholesterol, platelet aggregation, reduce oxidative stress and lowering blood pressure. In addition, consumption of red palm oil in daily use increases the efficiency of nutrient absorption, activates enzymes that play a role in liver metabolism, facilitates the formation of red blood cell hemoglobin, and improves immune function.

Carotenoids have important physiological activities such as a precursor of vitamin A, anticancer activity, immune boosting, anti-obesity, as well as an antioxidant that may help prevent degenerative diseases. One form of carotenoids is ß-carotene, which function plays a role in inhibiting the formation of free radicals. Free radicals can trigger lipid oxidation, which malonaldehyde as the end product is one indicator of oxidative damage to unsaturated fats and indicators of the presence of free radicals.

Free radicals also sparked enzymatically involve enzyme xanthine oxidase which produces reactive oxygen species, the superoxide radicals and hydrogen peroxide. The content of ß-carotene and tocopherol in palm oil is a unique source of antioxidants because of its ability to counteract lipid oxidase. Supplementation of vitamin E from palm oil has been reported to reduce the formation of gastric lesions in rats by oxidative stress. It is associated with the ability to inhibit elevated levels of adrenaline and nonadrenalin, as indicated by a reduction in the activity of the enzyme xanthine oxidase and xanthine dehydrogenase.

This study aimed to investigate the effect of consumption of crude palm oil on plasma ß-carotene, malondialdehyde (MDA) levels and xanthine oxidase activity on healthy housewives.

MATERIALS AND METHODS

MATERIALS

The main materials used were crude palm oil made by the production team of the Faculty of Agricultural Technology IPB, Bogor, Indonesia. Production of products with the brand “Sawita” held in Technopark IPB with registration number P-IRT No 207320101871. Ingredients for blood plasma analysis were: ethanol 95%, petroleum ether, ß-carotene standard (Sigma C4582-5mg), HCl, MDA standard (1,1,3,3 tetraetocsipropane), trichloroacetic acid.
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(TCA), thiobaturic acid (TBA), distilled water, BHT, aquabides, tris, HCl, CuSO₄, xanthine (Sigma-Aldrich X0626), glycine, coomasie blue, 85% phosphoric acid, bovine serum albumin (BSA).

Tools
Equipment used for blood plasma analysis include a 5 mL vacutainer tube containing EDTA, venoject and pump, siring 10 mL, 0.45 μm nitrocellulose membrane, laminar air flow, pasteur pipette, 15 mL valcon tube, freezer, waterbath, centrifuge, scales. analytics, 100 to 1000 μL micropipets, vortex, Shimadzu UV-Vis 1800 spectrophotometer, pH meters, and other glassware sets.

Criteria of subjects
Blood samples from 22 subjects were taken twice, before and after consumption of crude palm oil. The criteria of the subjects were aged 29-44 years (TABLE 1), having a healthy body based on medical examinations at the clinic, not pregnant or breastfeeding and not smoking. Considerations for selecting female subjects relating to the determination of food in the home menu which is still dominated by the mother/woman. They acted as nutritional gatekeeper, that someone in the household who acted as a decision maker to buy up to prepare food for the family. Selection of subjects housewife was aimed to minimize the diversity, because all subjects had almost the same activity. Subjects also live in the same area, so that activities and types of food were also not much different, so it was expected that the nutritional conditions of all subjects were also not much different. Subjects who were willing to have their blood drawn filled out informed consent and health information that has been informed in advance by medical personnel.

Intake of blood and blood plasma isolation
Blood samples were placed in a sterile vacutainer containing EDTA anticoagulants. Further more, the blood was centrifuged at a speed of 1500 rpm for 10 min to obtain of plasma, erythrocytes and the buffy coat layer. The isolated blood plasma was then stored in a refrigerator at -20°C until analysis.

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Intervention of subjects with crude palm oil

Intervention with palm oil for subjects were carried out after three times of meeting i.e. before the intervention, one month after the intervention, and two months after the intervention. The product was given after the first meeting, with a measure of the use for sautéing food, added to the finished food product, or other variations according to family habits at home. After that, an evaluation was done by filling out the questionnaire, with direct interviews to respondents. Monitoring was carried out at least once or twice a week, so that the consumption of crude palm oil can be controlled by researchers.

Blood plasma analysis

Blood plasma analysis included measurement of β-carotene levels using Neeld and Pearson methods,\textsuperscript{10,11} malondialdehyde,\textsuperscript{12} and xanthine oxidase activity.\textsuperscript{13} The instrument used for the analysis of these three parameters is the Shimadzu UV-Vis 1800 Spectrophotometer. Blood plasma analysis results were statistically analyzed using paired t-test. A p value <0.05 was considered significant.

RESULTS

Characteristics and observations of respondents

The observations showed 20 subjects were housewives, and 2 other subjects worked as traders (fried food/stalls). Based on the results of interviews, food preparation was conducted by raw preparations for fresh vegetables, steamed or boiled for carbohydrate dishes, stir-fried for vegetable dishes, fried for side dishes, and baked for snacks. Most subjects preferred to prepare it by frying it. This means that the use of oil in the family was quite widely used, and this shows that respondents’ knowledge about processing healthy food was still lacking.

Based on the results, subjects consumed crude palm oil everyday. The consumption method was conducted by adding to finished dishes, adding to fried dough, used for sautéing, frying eggs, or added to instant noodles. Subjects did not show any form of interference with the color, taste and smell of crude palm oil, because most were applied to saute.

Total consumption of crude palm oil

The effect of distributed crude palm oil consumption on the health of the respondent could be measured through the analysis of the respondent’s blood plasma. Based on the monitoring of the respondent’s family drawn by blood, the average amount of crude palm oil consumed by the respondent drawn blood was ± 3.58 mL per day. If multiplied by the amount of β-carotene found in crude palm oil products,\textsuperscript{14} which was 664.17 ppm, the amount consumed by the respondent was ± 2.38 mg of β-carotene per day. This amount was calculated based on the number of crude palm oil products distributed and the number of respondents’ family members divided by the duration of the intervention.

Plasma β-carotene levels

The β-carotene levels of each the healthy housewives (A-V) before and after 2 months consumption of crude palm oil are presented in FIGURE 1. The mean plasma β-carotene levels after crude palm oil consumption (1.965 ± 0.762 μmol/L) was higher than that before consumption (1.907 ± 1.006 μmol/L) although it was not significantly different (p>0.05). Never theless, the increase of the β-carotene levels was observed occurred in 16 subjects or around 72.3% of all respondents, with an average increase of 0.457 μmol/L or 28.3%. The increase of plasma β-carotene levels varied between individuals.
FIGURE 1. β-carotene levels of 22 healthy housewives (A-V) before and after 2 months consumption of crude palm oil.

**Plasma MDA levels**

Plasma MDA levels was determined based on the MDA-TBA method using an aspec photometer. FIGURE 2 shows the MDA levels of each healthy housewives (A-V) before and after two months consumption of crude palm oil. The mean plasma MDA levels after crude palm oil consumption (0.408 ± 0.190 nmol/mL) was lower than that before consumption (0.408 ± 0.190 nmol/mL) although it was not significantly different (p>0.05).

FIGURE 2. MDA levels of 22 healthy housewives (A-V) before and after 2 months consumption of crude palm oil.
Plasma xanthine oxidase activity

The plasma xanthine oxidase activity of each the healthy housewives (A-V) before and after two months consumption of crude palm oil are presented in FIGURE 3. The mean plasma xanthine oxidase activity before and after crude palm oil consumption were 3.458 ± 1.782 and 3.577 ± 1.939 U/g protein, respectively. There was not significantly different in the plasma xanthine oxidase activity before and after crude palm oil consumption (p>0.05). Ten subjects tended to decrease in xanthine oxidase activity, 11 respondents tended to increase, and one respondent was constant.

![FIGURE 3. Xanthine oxidase activity of 22 the healthy housewives (A-V) before and after 2 months consumption of crude palm oil.](image)

DISCUSSION

**Total consumption of crude palm oil**

Total the crude palm oil consumption was ± 3.58 mL daily. This amount is higher than that recommended by the National Health Institute of the United States that recommended 0.5 – 1.5 mg for daily consumption of vitamin A. However, vitamin A from carotenoids is relatively not toxic, therefore over consumption of the vitamin A is very safe. The upper limit of consumption of vitamin A tolerated for adults was set at 3000 µg/day in the form of preformed vitamin A.15

**Plasma β-carotene levels**

β-carotene is one type of carotenoids with highest provitamin A activity compared to other types of carotenoids. β-Carotene also has effective antioxidant activity as a free radical scavenger.15 Among 22 subjects involved in this study, six subjects showeded β-carotene levels decrease. The decrease is likely due to β-carotene has been converted to vitamin A and detected as retinol in blood plasma. Therefore β-carotene levels in blood plasma cannot be used as indicators of determining the vitamin A status of respondents. Serum/plasma carotene levels generally reflect consumption so that their values can vary. This is because there is no secretion from the β-carotene storage area to keep the concentration in the serum constant as in retinol. Carotene absorption depends on the amount consumed, carotene source and between individuals. The more carotene, the efficiency of converting carotene to vitamin A decreases. The absorption
efficiency will be higher if the amount of carotene consumed is small, and the absorption of carotene found in fats or oils is much better compared to the carotene contained in vegetables. The percentage of \( \beta \)-carotene absorbed by the human body varies between 8.7-65\%.\textsuperscript{16} The efficiency of converting \( \beta \)-carotene vitamin A is reduced by increasing the amount/dose of consumption. This explains why vitamin A poisoning does not occur in individuals who consume large amounts of \( \beta \)-carotene.\textsuperscript{17}

Of the six subjects who experienced a decrease in \( \beta \)-carotene levels, there was 1 respondent who experienced a drastic decrease, namely the N respondents. Research in the same respondent also showed a decrease in plasma retinol levels to levels below normal levels.\textsuperscript{18} This is thought to occur due to inflammation in the respondent. This assumption was also proved in other studies\textsuperscript{19} that used the same research subjects who stated that the respondent had an increase in C-reactive protein (CRP), an inflammatory marker protein. Some factors that can affect the absorption, bioconversion and bioavailability of carotenoids as provitamin A include consumer characteristics (e.g. metabolism and limits of absorption by the body), dosage, food fat content, food nutrient content, how to cook and prepare food, and other types of carotenoids in food.\textsuperscript{20}

**Plasma MDA levels**

The palm oil tocotrienol fraction has a protective effect from protein oxidation and lipid peroxidation after strenuous exercise. In addition, the antioxidant ability of palm oil \( \gamma \)-tocotrienol prevents an increase in blood pressure by decreasing lipid peroxidation and increasing antioxidant status.\textsuperscript{21} Supplementation of the palm oil tocotrienol fraction significantly reduced oxidative stress in the pancreas of diabetic rats as evidenced by a decrease in MDA levels and protein carbonyl.\textsuperscript{22} Supplementation of palm oil and melon egusi in rats fed high cholesterol for six weeks showed an increase in liver and serum lipid profiles and decreased serum and liver MDA levels compared with rats that were not treated with high cholesterol foods.\textsuperscript{23} The decreased of plasma MDA levels indicated inhibition by antioxidants. The plasma MDA levels did not show an increase or decrease in two respondents, or 9% of the total respondents.

The increase in MDA levels occurred in seven respondents, or 32% of the total subjects (FIGURE 2). It is associated with an imbalance of the amount of antioxidant intake that enters the body of the respondent with oxidative stress received by the respondent, which can also be influenced by the frequency of product consumption. The effect of product consumption is evident in subjects L, P, and S who after conducting interviews turned out to have never consumed crude palm oil several times, with the excuse of forgetting or not cooking. However, these factors cannot be used as a benchmark, because the high and low plasma MDA levels are highly dependent on the antioxidant status in the body. MDA is measured to assess lipid peroxidation due to an increase in oxidative stress in the body.\textsuperscript{24} Certain carotenoids which have a special chemical structure are able to neutralize or extinguish free radical activity, especially oxygen singlets by dissipating energy to all carotenoid molecules. In order to quell the oxygen singlet, the carotenoid molecule must have at least nine double bonds with a single bond between the double bonds. This arrangement is called a conjugated double bond, where \( \beta \)-carotene has that bond. The energy from the oxygen singlet is transferred to \( \beta \)-carotene and released to all single and double bonds, then released as heat and the \( \beta \)-carotenemolecule will return to its original form. At that time the oxygen
singlet has been converted to normal oxygen.

Carotenoids can affect the formation of lipid peroxidation processes in both the initial product such as conjugated diene or hydroperoxide and the final product in the form of MDA and TBARs. The binding of radicals in vivo will be related to the prevention of several diseases that occur due to free radicals. Consumption of carotenoid-rich foods such as fruits and vegetables can reduce the risk of developing certain types of cancer. Epidemiological studies and clinical trials reported that supplementation with sufficient carotenoids significantly reduces the risk of some disorders caused by the formation of ROS. The beneficial effects of carotenoid administration have been confirmed in several cancers, cardiovascular disorders, phosphor sensitive abnormalities, and eye disorders.25

**Plasma xanthine oxidase activity**

Xanthine oxidase is an enzyme that reacts at the end of the purine catabolism process, which catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid. Under normal conditions, xanthine oxidase is in the form of a precursor, xanthine dehydrogenase (XDH). Under stress conditions, such as ischemia and hypoxia, XDH undergoes sulfhydryl oxidation or proteolytic modification to xanthine oxidase.26 The increase in the action of the enzyme xanthine oxidase begins with increased activation of xanthine oxidase as a catalyst that produces superoxide radicals and hydrogen peroxide so that it triggers prooxidant conditions.7 Increasing the substrate in the form of xanthine will increase the change in the XDH which, in normal circumstances, is more physiological, becoming xanthine oxidase. Changes in these enzymes use more molecular oxygen than NAD+ as electron capture, so that in the further process will cause the formation of superoxide and hydrogen peroxide anions.7 Hydrogen peroxide can further react with metal ions (such as Fe2+) in the Fenton reaction or react with O2- and OH- in the Haber-Weiss reaction. Furthermore, the results of these reactions will eliminate the balance between the body's antioxidant and prooxidant status, giving rise to oxidative stress, which is characterized by the occurrence of lipid peroxidation.27

Plasma xanthine oxidase is still below the normal value of previous studies conducted, where the average xanthine oxidase activity in healthy subjects (aged 40-65 years) who were not given stress treatment was 9.6 U/g protein. This difference in value can be caused by the age of the respondent. In this study, the age of subjects drawn blood between 29-44 years.

Previous study28 showed that the xanthine oxidase activity increase in related with age. The low activity of xanthine oxidase can be caused by subjectshaving normal antioxidant status. Another suspicion that oxidative stress caused by exposure to free radicals has been counteracted by antioxidant enzyme in the body and the presence of exogenous antioxidants (ß-carotene and tocopherol). Supplementation with vitamin E extracts of palm oil and α-tocopherol decreases the formation of gastric lesions, which are associated with their ability to inhibit stress caused by increased levels of adrenaline and non-adrenaline, and through decreased activity of xanthine oxidase and XDH.8

This study demonstrated that crude palm oil consumption did not affect the xanthine oxidase activity. This is presumably because the blood drawn from the subjects with good health status. However, it can be explained that the antioxidants in crude palm oil can suppress the activity of the xanthine oxidase.
CONCLUSION

This study demonstrated that consumption of crude palm oil can increase plasma β-carotene levels of healthy housewives. Crude palm oil can be an alternative natural food source of provitamin A due to the high β-carotene. Further study will be carried out to evaluate the effects of crude palm oil consumption on subjects at risk or with heart disease, hypercholesterolemia, gout, smokers or other infectious diseases.

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