The correlation of some of the heating of various palm oils to histologic and liver function of rats (*Rattus norvegicus*)

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Abstract. Repeated heating of palm oil will result in increased peroxide values and can cause disease in the organisms that consume them. The study used a completely randomized design with 15 treatments (five types of palm oil cooking with three types of heating/one time, four times, and eight times) and five replications. Results of data analysis of test parameters such as SGOT and SGPT values, histologic liver damage showed significant differences (p<0.05). There has also been a significant correlation (p<0.05) between recurrent heating of palm fried oils with liver histologic damages and liver function values.

1. Introduction

The Indonesian population consumes per capita cooking oil in 2011 is 8.24 liters/capita/year. There is a high tendency for people to reuse cooking oil in cooking to save on food preparation costs [1]. This practice is detrimental to health as oil is repeatedly heated to undergo a series of chemical reactions known as thermal oxidation. The thermal oxidation of heated oil products triggers free radicals that are pathogenetic to various diseases including hypertension [2]. Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidant systems capable of inducing lipid peroxidation and free radical formation. Oxidative stress and lipid peroxidation have been heavily involved in the pathogenesis of hypertension and atherosclerosis [4-6].

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peroxidation which then results in cell membrane damage with morphological and biochemical changes followed by cell function impairment and termination of hepatocyte death [6].

Some reports suggest that consumption of trans fatty acids results in health problems, such as increasing total cholesterol, LDL, lowering HDL cholesterol and increasing the total cholesterol ratio [7], endothelial disorders [8], insulin not sensitive [9,10], and improved tumor necrosis factor (TNF) and C-reactive protein [11]. In addition, the consumption of trans fat causes a person at high risk of diabetes [12] and /CHD [13]. Another study, states that a high-fat diet with different levels of trans fatty acids can induce oxidative stress and liver dysfunction in mice [14]. Martianto et al. [15] in Makassar showed poor and non-poor people using the same cooking oil to fry twice as much as 61.2%, tree times 19.6% and four times 5.4%.

In this research simulation of frying with technique commonly used in household, that is by using cooking oil until eight times and oil remain silent in frying until used for subsequent frying. Furthermore, observations on oil and products for the safety evaluation are consumed. It coronary heart disease is expected that the results of this study can be the basis for households in using cooking oil as well as scientific information generated when cooking oil is used repeatedly.

2. Methods

This research is true experimental research with completely randomized design. The research was conducted at the Animal Physiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The subjects of the study were white rat wistar, rats aged eight weeks with weight between 180-200 g and with healthy condition. Independent variables of this research are five types of palm cooking oil and heating of the one time, four times, and eight times. While the dependent variable is the lipid profile of SGOT and SGPT values, as well as the condition of the liver tissue. The sample size refers to the Federer formula [16]; (t-1) (n-1) ≥ 15, t=3x5, n=5, then the total number is 75 heads. All rats were randomly divided into five study groups: the types of palm oil (A, B, C, D, and E) and three recurrent heating groups (one time, four times, and eight times).

Provision of palm oil (one time, four times, and eight times) in the A, B, C, and D group of 2.5 ml/200g BB peroral per day for 30 days then examined levels of SGOT, SGPT and liver histology. The frying process begins by incorporating fresh cooking oil into the frying pan as much as ± 1 liter, then heated to 185°C and then 100 g meat fried until cooked for five minutes.

Data were analyzed using computer program and tested statistic with Anova test of 5% level at bootstrapping. Different test of two treatment groups did not pair with posthoc test if anova result p<0.05 to see difference of SGOT and SGPT levels, and histology of liver after treatment in each group was done.
3. Results and Discussion

3.1 Blood Analysis (SGOT and SGPT)

Based on the measurements that have been done in accordance with the treatment groups, the results obtained as in Figure 1, Figure 2, Figure 3, and Table 1.

![Figure 1](image.png)

**Figure 1.** Table of observation results of SGOT blood serum of mice after administration of packaged palm oil (A, B, C, D, and E) and repetition of heating (1x, 4x and 8x).
Figure 2. RBC serum blood serum observation results after the provision of packaging palm cooking oil (A, B, C, D, and E) and repetition of heating (1x, 4x and 8x).

In Fig. 1 and 2, there was a significant effect on the increase of SGOT and SGPT on all types of packaged palm oil starting and recurrent heating (p<0.05). The increase in serum SGOT was followed by a marked increase of SGPT (p>0.05) as it repeated the heating of cooking oil. It is generally concluded that there is a negative influence of repeated heating of packaged palm oil.

This proves the difference in quality of packaged oils as well as the negative effects of recurrent heating. Repeated warming can cause oil peroxide numbers to negatively affect blood lipid profile (increased total cholesterol and LDL and decreased HDL of blood) may even cause hyperlipidemia. Chronic hyperlipidemia will cause LDL buildup, so that it can be oxidized by the effects of free radicals that activate nitric oxide. This oxidized LDL, swallowed by macrophages through scavenger receptors, forming a foam cell. This foam cell that will form aggregate and will eventually form a fatty streak. Thus, the fatty streak can be an accumulation of intracellular smooth lipid lipid and accumulation of smooth muscle-extracellular lipids as well as further atherosclerosis with the formation of surface defects, hematomas and thrombus [17,18].

Oxidative stress e.g. reactive oxygen species (ROS) caused by an increase in total cholesterol and LDL and decreased HDL of blood will interfere with endothelial vasodilatation which then increases reactivity and vascular resistance. Reactive oxygen species can increase the absorption of peroxinitrit oxide which is also a free radical. Furthermore, ROS causes inflammation of blood vessels and activation of growth signal pathways [19, 20]. There is a report stating there is a relationship between stress oxidative and hypertensive patients. Patients with hypertension have been reported to have high levels of malondialdehyde (MDA) and are a product of lipid peroxidation breakdown and lower antioxidant activity. Recent studies have shown a positive linear correlation between blood pressure and oxidative stress [21]. On the other hand, there is a negative correlation between blood pressure and antioxidant
activity [22]. Previous studies have also been reported that heated oil causes increased lipid peroxidation and increases the risk of atherosclerosis [23, 24].

In normal circumstances SGOT and SGPT enzyme levels in the blood are low because they are present in the cell, but if tissue damage occurs, the cells will break and the enzymes will break down from the hepatocytes into the circulatory system, so that the levels in the blood will increase compared with normal [25]. Normal values of the human serum Glutamic Oxaloacetic Transaminase (SGOT) range from 3-45 units per liter (u/l), whereas the SGPT (Serum Glutamic Piruvate Transaminase) considered normal is 0-35 units per liter (u/l) [26]. According to Harrison [27] the normal SGOT and SGPT in humans is about 35 U/I while the normal SGOT level of white mice is 141 ± 67.4 IU/I and the normal SGPT level of white rat is 12.6 ± 4.40 IU/I [28]. High levels of SGOT-SGPT as a test/liver function test are not always marked by high hepatocyte damage as it depends on the extent, type of hepatic damage, the sensitivity of the test method and the presence or absence of compensatory efforts by healthy liver cells. According to Sudoyo [29] there is often no relationship between high levels of enzymes with degrees of hepatocyte damage. In acute hepatistic cases, with little damage to hepatocytes there can be a tremendous increase in SGOT and SGPT enzymes [30-35]. Increased SGPT greater than SGOT in case of acute infection, but not until cell mitochondria. If mitochondrial damage or parenchymal damage (hepar) is seen to increase is SGOT.

3.2 Histopathological observation (index necrosis) of rat liver

Based on histopathologic observation from mouse liver, the result of the research as shown in Table 1 and Figure 3 below.

Table 1. Histopathological observation (index necrosis) of rat liver after packed cooking oil (A, B, C, D, and E).

| No. | Type of Palm Oil | Repetition of frying/heating * | 1x     | 4x     | 8x     |
|-----|-----------------|-------------------------------|--------|--------|--------|
| 1.  | A               | 0.41±0.08<sup>a</sup>         | 0.91±0.05<sup>b</sup> | 1.57±0.35<sup>c</sup> |
| 2.  | B               | 0.43±0.10<sup>a</sup>         | 0.93±0.09<sup>b</sup> | 2.08±0.07<sup>d</sup> |
| 3.  | C               | 0.53±0.03<sup>a</sup>         | 1.23±0.08<sup>b</sup> | 2.04±0.14<sup>d</sup> |
| 4.  | D               | 0.91±0.15<sup>a</sup>         | 1.01±0.03<sup>b</sup> | 2.39±0.08<sup>c</sup> |
| 5.  | E               | 0.62±0.13<sup>a</sup>         | 1.07±0.10<sup>b</sup> | 2.44±0.02<sup>c</sup> |

Description: * / ** = there is no significant difference between the oil type and heating reactions ie. p = 0.161 (<sup>a</sup>p>0.05).
Figure 3. Histology of rat liver after administration of packaged palm oil (A, B, C, D, and E). black arrow = central vein, yellow arrow = necrosis cell. Magnification; 400x.

Metabolic disorders in hepatocytes due to toxic substances that cause morphological or functional damage can be overcome by the regeneration of hepatocytes. The low SGOT-SGPT enzyme does not necessarily indicate damage to hepatic cells [36-39]. This is based on the statement Arnita [26] because SGOT is not only generated on the liver alone, but rather found in the heart organ, liver, skeletal muscle, pancreas, lung, red blood cells and brain cells. When the organ cells are damaged, the SGOT will be released in the blood. According to Horrison [27,30,31,32] SGPT is found specifically in the cytosol, whereas SGOT is found in mitochondria and cytosol.

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