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Utilization of *Anadara antiquata* shells to improving of waste cooking oil based lipid profiles measurements in experimental rats

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ABSTRACT

Waste cooking oil (WCO) was purified from the impurities to improve the quality based on lipid profile measurement of biochemical serum parameters of experimental rats. The bio sorbent used are the shells powder of

Anadara antiquata. The optimum condition of used oil purification was achieved with 15 g bio sorbent dose and 2 weeks of immersion. After purification process, the experimental rats were administered with the *A. antiquata*-treated (WCO) orally for 2 weeks. The measurement result of lipid profile indicate that administration of bio sorbent-treated used oil cannot reduce the level of malondialdehyde (MDA), even there is an increase in the percentage of MDA as 18,67%, but it is lower if compared to WCO-treated rats as 29,79% in increasing of MDA levels. There are no significant changes in total cholesterol on *A. antiquata* treated wasted cooking oil, compared with WCO treated rats that there is increased of LDL levels as 11,47% compared to untreated rats. The increased of LDL levels in group *A. antiquata* treated WCO was 4,75%, which far lower than the increase in group WCO treated rats which is 43,12%. It can be concluded that *A. antiquata* has potential as adsorbent to improve the quality of lipid profile of waste cooking oil and oxidative stress

Keywords: *Anadara antiquata*, lipid profile, used cooking oil

INTRODUCTION

In modern society, oil is a primary requirement in preparing the various of food, where the oil is used to frying the food to increase the taste and give better presentation of the food. as this method becomes increasingly popular, accumulation of waste generated from cooking oil also increased [1]. During the frying process, oil undergoes many physical and chemical changes. These changes after prolonged cooking make the oil unfit for human consumption, because it could lead to serious health problems such as gastrointestinal disorder and even mutagenesis in human body. Waste cooking oil (WCO) could also lead to serious environment problems such as bad odour. It is said that one liter of oil that poured into natural water bodies could contaminated 500.000 L of water [2]. When the oil is heated, it will form hydroperoxide and aldehydes, that will absorbed into the fried food. One of the product is malondialdehyde (MDA) which is a marker of lipid peroxidation. In addition, the repeated heating of the frying oil will lead to the formation of free radicals that are harmful to health [3]. Consumption of repeatedly heated cooking oil might increase the risk of developing atherosclerosis. Lipid peroxidation products induce oxidative stress in endothelial cells, resulting in endothelial dysfunction that could eventually lead to the formation of atherosclerosis. Consumption of repeatedly heated cooking oil is also associated with increasing in total serum lipid and low density lipoprotein (LDL) [4]. The recycling or purification using natural adsorbent has been studied. Girsang et al [5] reported that powder skin of *Salacca zalacca* could improve the quality of waste cooking oil by reducing the MDA value and lipid profile (total cholesterol and LDL). Munaf et al [6] also reported that the seed powder has potential as bio sorbent. It could remove the cadmium, copper and zinc ions as 62,79%, 90,69% and 63,09% respectively in aqueous solutions. In this study, we reported the potential ability of shell powder of *Anadara antiquata* for purification of waste cooking oil and effect of administration of *A. antiquata*-treated WCO in lipid profile of experimental rats.

MATERIAL AND METHOD

Chemicals and equipment

All chemicals used in this experiment are analytical grade, obtained from Merck (Germany). A cruiser (Fritch, Germany), analytical balance (Kern & Sohn, GmbH), rotary shaker (Edmund Buhler 7400 Tubingen), UV-Vis (Thermo Insight), FTIR (Nicolet iS10 with KBr) and SEM (Hitachi S-3400N) and light microscope were used in this experiment

Waste Cooking Oil

Waste cooking oil that used in this experiment was chicken/fished used oil, obtained from 'Pecel Lele' food stall in Padang city. The new oil was considered as standard

Powder of A. antiquata

Shells of *A. antiquata* obtained from local market trader in Medan. The shells first washed with running water until clean and then grinded using a grinder into a powder form. This powder the air dried at room temperature for 2 days.

Preliminary test of improving quality of oil color

The powdered *A. antiquata* shells was weighed as 5, 10, 15 and 20 g in a beaker glass. Then in each beaker glass was added 50 mL WCO. The beaker glass then wrapped with aluminium foil and allowed to stand for 2 weeks without stirring. After 2 weeks the WCO the filtered using gauze and the change of the color was observed visually.

Optimization of immersion time on the quality color of WCO

The optimum dose of *A. antiquata* from previous pre eliminary test was weighed and put in 3 beaker glass. Then in each beaker glass was added 50 mL of WCO, and allowed to stand for 1 week, 3 weeks and 4 weeks without stirring. The WCO was filtered and the change of the color was observed visually.

Experimental Design of Lipid Profile Analysis and Histopathology

9 rats were divided into groups (group 1, 2, and) with 3 rats in each group. The rats in each group were administered with 0,5 mL/200 g bw new cooking oil (NCO) in group 1, WCO (group 2), *A. antiquata* treated- WCO (group 3) for 15 days orally. In the last day of experiment, the rats were anaesthetized using chloroform and sacrificed. The blood was collected for lipid profile analysis (MDA, total cholesterol, LDL, and triglyceride). The hearts of the rats were dissected and preserved in Bouin's solution for histopathology analysis.

RESULT AND DISCUSSION

Pre eliminary test of improving quality of oil color

After immersion with powdered *A. antiquata* for 2 weeks, the WCO seemed to change color from dark yellow oil into brighter color, as shown in figure 1

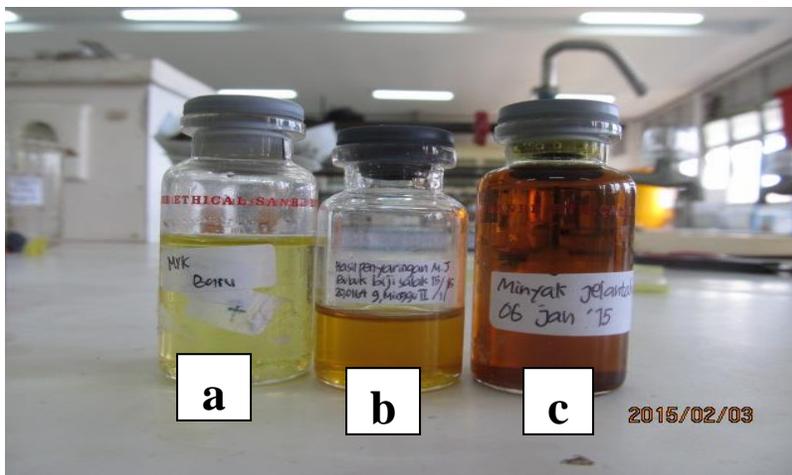


Figure-1: Improved color quality of WCO; a. new oil; b. *A. antiquata*-treated WCO; c. WCO

Based on the level of turbidity it was concluded that the optimum for purification of WCO were after immersion for 2 weeks at a dose 15 g of bio sorbent. The resulted turbidity levels indicate the ability of powdered shell of *A. antiquata* to increase the physicochemical quality of WCO. The principle of purification or turbidity of WCO using adsorbent was elimination of a series of contaminants which comprise for example phosphatides, free fatty acids, pigment, odour and flavour (including aliphatic aldehyde and ketone), waxes as well as trace metals. Vishnuprasad and Kumar [7] reported that low cost adsorbent such as crab shell is newly recommended as good adsorbent because it can be used as a coagulant, reducing agent for suspended solid and also reduce the turbidity the waste water.

Oxidative stress marker and lipid profile analysis

The level of MDA in experimental rats from each group were shown in figure 2

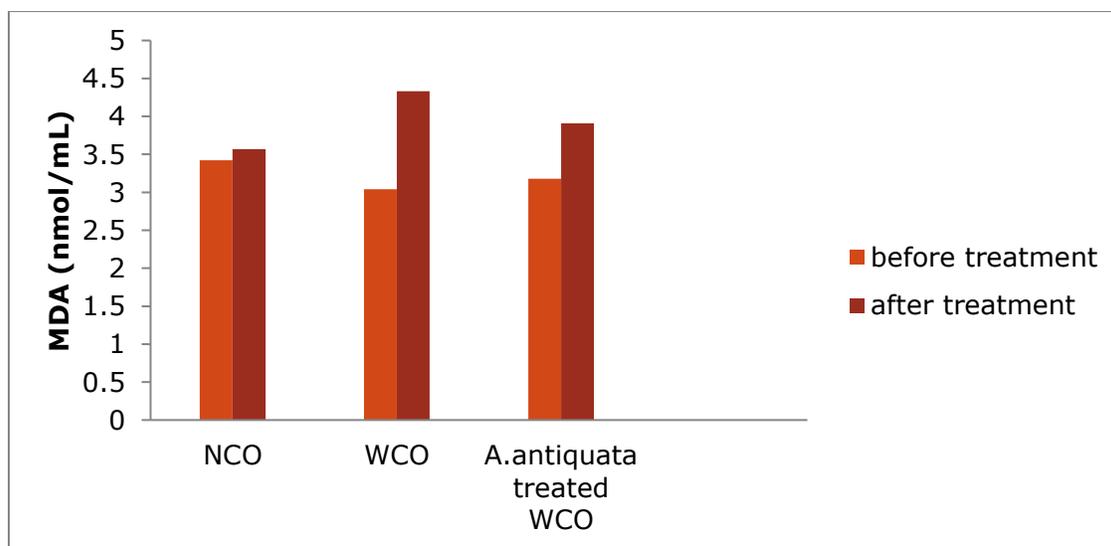


Figure-2: Effect of administration of group 1 (NCO), group 2 (WCO) and group 3 (*A. antiquata*-treated WCO) on MDA level in experimental rats

There are elevated levels of MDA in group 1, 2 and 3 after administration with new oil, WCO and *A. antiquata*-treated WCO as 4,20%, 29,79% and 18,67% respectively. MDA is one of lipid peroxidation product, so the measurement of MDA could be used to examine the levels of lipid peroxidation [8]. Zein et al [9] in their study about improvement quality of used cooking oil by rice husk ash (RHA), was reported the similar result. Zein et al reported that MDA levels new palm oil (before used) is 4,85 nmol/L, MDA of used palm oil was 6,91 nmol/L. The rice husk ash could reduce the level of MDA from 6,25 to 5,55 nmol/L with increasing the value of adsorbent mass from 5 to 20 g. During the oil-frying process of food preparation, various chemical reactions occur, such as oxidative and hydrolytic degradation and polymerization. The ingestion of oxidized frying oil increases the consumption of antioxidants in living tissues and lead to increase of oxidative stress markers [10]

Total cholesterol analysis

The effect of administration of cooking oil on total cholesterol of experimental rats was showed in Figure 3

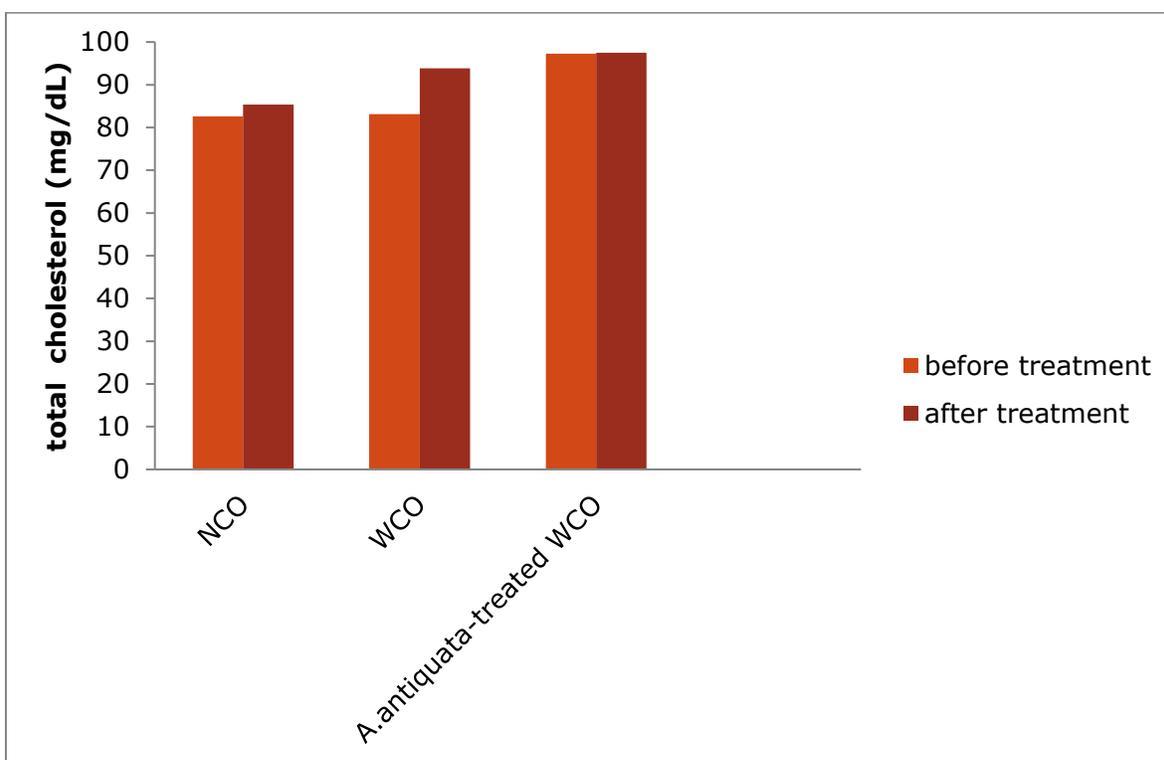


Figure-3: effect of administration of cooking oil on total cholesterol levels in experimental rats

Based on Figure 2, there was an increase in total cholesterol in group 1 (NCO), group 2 (WCO) and group 3 (*A. antiquata*-treated WCO) as 3,24%; 11,47% and 0,21%. Based on this data, the highest increasing of total cholesterol was on group 2 and the lowest was in group 3. El-Bialy et al [11] reported that the lipid profile of experimental rats showed a significant increase in serum total cholesterol significantly particularly in RBO (repeat boiled oil) than OBO (one time boiled oil). The oxidation process of heated oil changes in fatty acid configuration from the cis isomer to the trans. Intake of trans fat was found to be correlated with the increase in serum total cholesterol and LDL levels. There was no significant increase in total cholesterol in group 3. This means *A. antiquata* was able to reduce trans-fat content in WCO.

Low Density Lipoprotein (LDL) Analysis

The effect of administration of cooking oil on low density lipoprotein (LDL) of experimental rats was showed in Figure 4

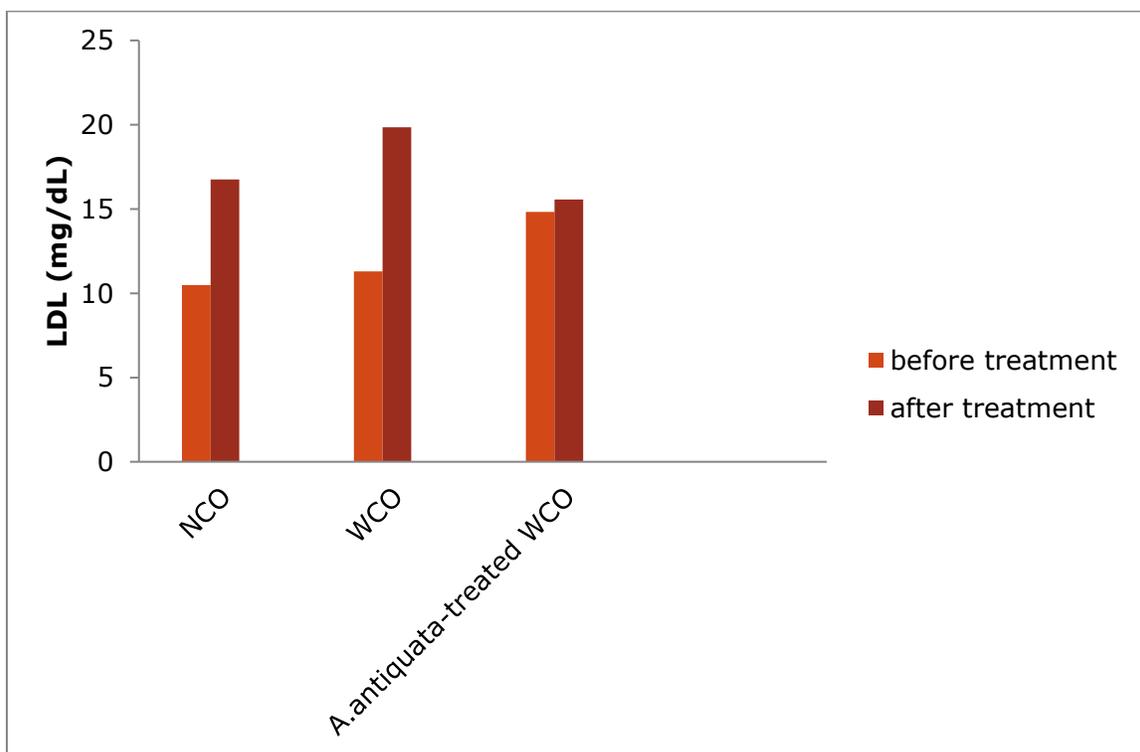


Figure-4: effect of administration of cooking oil on LDL levels in experimental rats

Figure 4 showed that there is increased level of LDL in group 1 (NCO), group 2 (WCO) and group 3 (*A. antiquata*-treated WCO) of 37,43%, 43,12% and 4,75% respectively. Based on this data, the highest elevated levels of LDL were occurred in rats in group 2. Jaarin et al [12] reported that repeating heated of oil at high temperatures ($\geq 180^{\circ}\text{C}$) result in the thermal oxidation of oil, which lead to changes in configuration of the fatty acid to change from cis isomer to trans isomer. This configuration changes cause the polyunsaturated fatty acids to acquire undesirable properties associated with saturated fatty acids, such as their correlation with increased serum cholesterol levels and low density lipoprotein (LDL). LDL conveys cholesterol from the liver to the cells, and if the LDL concentration in the blood is too high, it precipitates in the arteries. This leads to a risk of arterial diseases [13]. From the result, the administration if *A. antiquata*-treated WCO lead to the lowest levels of LDL, this means that *A. antiquata* was effective to reduce the LDL levels in WCO.

Histopathology of Heart Tissue

The histopathology analysis of cardiac tissue of experimental rats in each group was showed in Figure 5.

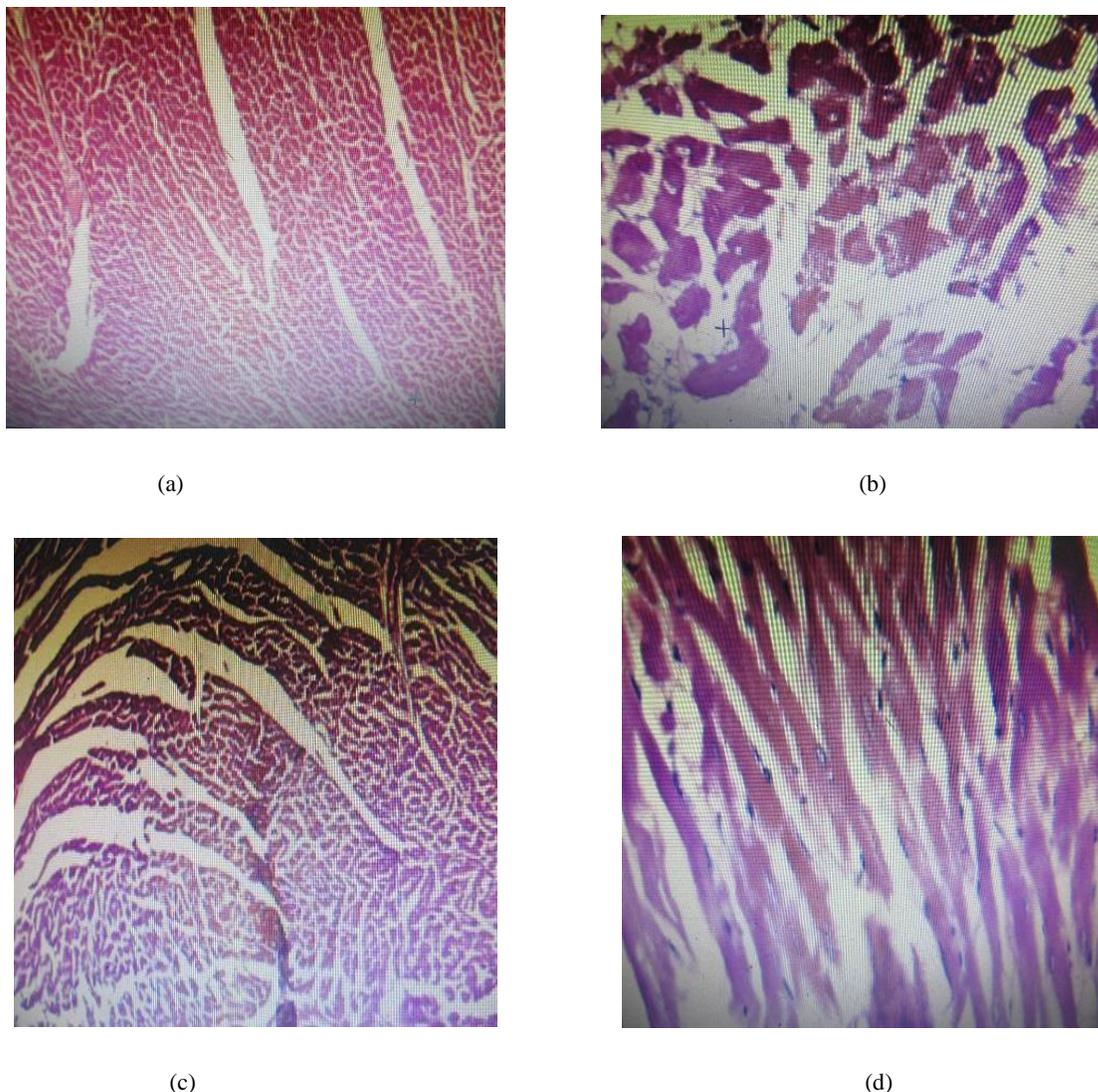


Figure 5. Histopathology analysis of cardiac tissue : (a) cardiac tissue of rat in group 1 (NCO), cardiac cells appear normal with normal blood vessels, (b) cardiac tissue of rat in group 2 (WCO), most of cardiac cells appear normal, some of the cells hypertrophied and the nucleus apparently missing, (c) cardiac tissue of rat in *A.antiquata*-treated WCO, cardiac cells appear normal but the blood vessels dilated, (d) cardiac cells in control rat (without treatment), cardiac tissue and coronary artery looks normal

The similar result was reported by Farag et al [14], where cross section of heart tissue of rats administered non-fried sunflower oil displayed no histopathological changes. Heart tissues of rats which is given fried sunflower oil heated for 20 h induced dilatation and congestion of myocardial blood vessel.

Repeated heating of the cooking oil makes it more susceptible to lipid peroxidation [10]. Lipid peroxidation generates a wide spectrum of volatile or non-volatile components, including free fatty acids, alcohols, aldehydes, ketones, hydrocarbons, trans isomer and epoxy compound. As a result, when the same cooking oil is re heated and reused, the chemical reactions that occur in cooking oil will enhance foaming, darkening of oil color and increased viscosity. Lipid peroxidation is the key of mechanism that will lead to cell injury and mutagenesis [15]. Lipids, particularly polyunsaturated fatty acids are key target of this mechanism

because they contain oxidizable double bonds. The unstable free radicals which will stabilize themselves by abstracting electrons from membrane lipids to initiate a self-propagating chain reactions that will lead to damage of the cells and tissues [16].

CONCLUSION

Based on the result it can be concluded that *A. antiquata* has potential as an adsorbent to improve the quality of lipid profile of waste cooking oil particularly in total cholesterol, LDL levels and oxidative stress marker, malondialdehyde.

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