



## THE EFFECT OF BEEF FAT INTERESTERIFICATION ON THE GUINEA PIG'S LIPID PROFILE

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### ABSTRACT

Properties of fats are determined by the composition and position (sn-1,2,3) of fatty acids in the fat molecule. Palmitic acid is one of the most atherogenic fatty acids in the sn-2. Interesterification will change the position of palmitic acid in the fat molecule. This study aimed to determine the effect of interesterification on the atherogenic properties of fat. The fat used was beef, extracted by *dry rendering*, and chemically interesterified using a Na-methoxide catalyst for 20, 30, 60, 90, and 120 minutes. The melting point of the fat was measured using a *melting point apparatus* then given to guinea pigs. For 21 days, the propyl lipid was measured using the enzymatic method using a micro lab 300 spectrophotometers. The melting point of beef fat from interesterification for 60 minutes was constant, meaning the reaction was complete. Interesterification of beef fat showed an increase in atherogenicity seen from the increase in total cholesterol, triglyceride, LDL and decreased HDL levels after interesterification, respectively  $69.20 \pm 20.24$ ;  $68.60 \pm 18.81$ ;  $40.43 \pm 15.18$ ;  $14.33 \pm 3.01$  mg/dl compared with no interesterification  $62.27 \pm 16.02$ ;  $62.87 \pm 14.08$ ;  $37.84 \pm 13.41$ ;  $11.80 \pm 2.15$  mg/dl (ANOVA,  $P < 0.05$ ).

**Keywords** : *interesterification, chemical interesterification, lipid profile, beef fat, atherogenic, palmitic acid, fatty acids*

### INTRODUCTION

Excessive fat consumption can form plaques that can weaken blood vessels and inhibit flow in blood vessels, so that blood circulation is obstructed, known as atherosclerosis. Atherosclerosis is a disease that occurs due to thickening and loss of elasticity of the artery walls—characterized by the presence of atheroma in the intima of arteries which contains cholesterol, lipids, and lipophages. Efforts to prevent and improve atherosclerosis include reducing plasma cholesterol levels (Suyatna, 1995). The atherogenic properties of fats depend on the length of the chain of saturated fatty acids that compose them and the position of these fatty acids in the fat structure (Triacylglycerol = TAG). Short-chain fatty

acids (C-4 to C-8), medium-chain fatty acids (C-8 to C-12), and unsaturated fatty acids are usually not atherogenic. However, saturated long-chain fatty acids, including myristic acid (C-14) and palmitic (C-16), are atherogenic, while stearic acid (C-18) is not because it will quickly be converted to oleic acid, so it is considered neutral (Silalahi and Nurbaya, 2011). There are three stereospecific positions of fatty acids (*stereospecific numbering* = sn), namely the sn-1, 2, and three positions on the fat molecule (TAG); human lipase enzymes work specifically at the sn-1,3 position and do not hydrolyze the product at the sn-2 position (Decker, 1996. Willis et al., 1998). The fat used in the manufacture of margarine can be derived from animal fat or vegetable fat. The animal fats

commonly used are derived from lard and beef fat, while the vegetable fats commonly used are coconut oil, palm oil, soybean seed oil, sesame oil, kapok oil, corn oil, and wheat germ oil (Winarno, 1995). According to Berry (2009), fat-containing palmitic acid comes from lard, beef tallow, and palm oil.

Palmitic acid in beef fat is in the sn-1 and sn-3 positions, with such a position that palmitic acid is not absorbed, so it is not atherogenic. Interesterification of beef fat causes a shift in the position of palmitic acid from the sn-1,3 position to the sn-2 position so that it becomes atherogenic (Silalahi and Nurbaya, 2011). Interesterification is a process to modify fats or oils, which causes changes in the composition and distribution of fatty acids in the triglyceride molecules. There are changes in properties that are different from the original (Silalahi, 1999). In the manufacture of margarine, this method is one of the processes used to avoid the formation of trans isomers (Petrauskaite, 1998). Chemical interesterification will result in randomization of the presence of fatty acids at each position in the glycerol molecule. Transfer or random exchange of acyl either in one molecule or between molecules of triglycerides will occur until an equilibrium state is reached (Ibrahim et al., 2008; Robinson et al., 2009), an ideal time is needed to achieve perfect interesterification, and of course, it will affect the atherogenicity of interesterified beef fat previous research conducted by Kritchevsky (2000), which is in lard almost all palmitic acid is at sn-2, so it is more atherogenic than tallow (beef fat) only 4% palmitic acid at the sn-2 position.

However, after interesterification (randomization), these fats contain 8% palmitic acid at the sn-2 position. The consequence is that the atherogenicity of lard decreases while that of tallow increases. Cottonseed oil randomization changed the palmitic acid from 2% to 10% at the sn-2 position. It was found that the atherogenicity increased threefold, palm oil contains 3% palmitic acid in sn-2, and after interesterification, it becomes 13.6% and increases atherogenicity by 34% (Silalahi, 1999; Kritchevsky, 2000; Silalahi, 2006).

Kritchevsky (2000) has proven that interesterification can change the atherogenicity of fats; however, no one has yet explained how long interesterification lasts so that it can change the

atherogenicity of these fats and oils, so it is essential for us to examine the effect of the length of chemical interesterification on the atherogenicity of beef fat and palm oil. by measuring changes in blood lipid profile levels using guinea pigs as experimental animals.

## **MATERIALS AND METHODS**

This study is an experimental study that compared beef fat's chemical interesterification time to changes in guinea pigs' blood lipid profiles. This research was conducted at the Research Laboratory of the Faculty of Pharmacy, Tjut Nyak Dhien University, and the Ministry of Health, North Sumatra Health Service, when the research was conducted for approximately (six) months.

### **Tools and Materials**

The tools used are a micro lab spectrophotometer 300 (vital scientific), centrifuge (swing type model CD-50 SR Tomy Seiko), rough balance, analytical balance (Metier Toledo), Melting Point Apparatus (Stuart), micropipette (Clinical ). Ice flask, 1 ml syringe, polytube, nail clipper, and other necessary tools. The materials used in this study were palm oil (bulk), beef fat, sodium pellets, methanol (Merck), benzene (Merck), citric acid (Merck), n-hexane (Merck), anhydrous Na sulfate, distilled water, cholesterol reagent (Dialab), triglyceride reagent (Dialab), Dialab HDL reagent). standard comparison reagent Diacon-N (Dialab), cholesterol standard reagent (Dialab), triglyceride standard reagent (Dialab), HDL standard reagent (Dia lab).

### **Sample Preparation The sampling**

the technique was carried out purposively at the lemonade market. The beef fat used is fresh fat taken from the belly (Arikunto, 1993). The fat tissue samples were washed, sliced into small pieces, and put into a beaker. Then the sample was put into a *dry oven* that had been set at a temperature (75°C) left for 6 hours until the fat tissue melted (Hermanto et al., 2008).

### **Chemical Interesterification Procedure**

A 150 ml sample was put into a flask, and 10 ml of 0.1 N NaOCH<sub>3</sub> catalyst was added. The mixture is then stirred with a *magnetic stirrer* at 4000 rpm for 30, 60, 90, and 120 minutes at a temperature of 60-70 °C. The reaction results are then neutralized

by adding a 20% (w/v) citric acid solution and then putting in a separating funnel and adding 150 ml of n-hexane, which was then washed three times with distilled water. The addition of anhydrous sodium sulfate then dries the top layer. It is filtered and rotated by evaporation to obtain the reaction products, then analyzed for melting points (Barus, 2007; Robinson et al., 2008).

### Melting Point Determination

To determine the melting point of beef tallow and palm oil and the interesterification results using the *Melting Point Apparatus* (Stuart): if it is in solid form, it is previously heated until it melts. The sample was put into a capillary tube with a diameter of 1 mm, then frozen for 24 hours in the freezer (Barus, 2007). A capillary tube is inserted into the apparatus, and the temperature is gradually and slowly raised until the fat melts and the melting point is recorded.

### Experimental Animals The

Animals used in this study were guinea

pigs (*Cavia cobaya*) weighing 300-500 grams. Before the experiment started, it was first kept in a suitable cage for two weeks to adapt to its environment (Directorate General of POM, 1995). The animals used in this study were 30 healthy and adult guinea pigs which were first quarantined for two weeks to adapt to their environment. Then the profit level of the initial blood serum lipid was measured.

### Testing the Effect on Lipid Profit of Marmot Blood Serum

Before testing, the guinea pigs were fasted (not eating but still drinking) for  $\pm$  18 hours. Then each guinea pig was weighed and marked. The guinea pigs were divided into ten groups, consisting of three guinea pigs, as shown in Table 1. The guinea pigs were given oil/fat 1 g/kg BW/day for 21 days orally. Furthermore, each group of guinea pigs was determined for their blood serum lipid profit levels on days 1,7,14, and 21.

**Table 1.** The treatment group is experimental animals.

group	Sample
A	Beef fat
B	fat oil interesterificated for 30 minutes
C	Fat oil cattle for 60 minutes
D	sap fat oil for 90 minutes
E	Interesterified beef cattle oil for 120 minutes
F	Palm oil
G	Palm oil interesterified for 30 minutes
H	Palm oil interesterified for 60 minutes
I	Coconut oil Interesterified palm oil for 90 minutes
J	Palm oil hash interesterified for 120 minutes

Then the guinea pigs have fasted for 10-14 hours. Blood was taken by cutting the nails. Then the dripping blood was accommodated

approximately 0.5 ml in the tube. The scar on the guinea pig's nails is closed using a cotton swab. The blood that has been taken is centrifuged for 10 minutes at a speed of 1000 rpm, 2 layers will be

produced, namely the serum and solids, the serum is pipetted (clear) and stored in the refrigerator at a temperature of 2-8 °C (Smith and Soesanto, 1988).

### Measurement of Total Cholesterol Levels First measure

the blank solution (1 ml of cholesterol reagent ± 10 l of distilled water), then measure the standard solution (1 ml of cholesterol reagent + 10 l of cholesterol standard solution. Then ten pl of serum is taken and mixed with 1 ml of cholesterol reagent, then put into the reaction float, then mixed until homogeneous. Incubated at room temperature for 20 minutes and then measured (Prangdimurti et al., 2007). Measurements using a 300 micro lab spectrophotometer with a wavelength of 546 nm. The results were recorded.

### Measurement of Triglyceride Levels

Measured first a blank solution (1 ml of triglyceride reagent + 10 ml of distilled water), then measured the standard solution (1 ml of triglyceride reagent + 10 l of standard triglyceride solution. Then 10 l of serum is taken and mixed with 1 ml of triglyceride reagent, then put into a test tube, mixed until homogeneous, incubated at room temperature for 20 minutes, and then measured (Prangdimurti et al., 2007). Measurements using a micro lab 300 spectrophotometers with a wavelength of 546 nm, the results were recorded.

### Measurement of HDL Levels

The supernatant solution was made first by mixing 200 l of standard HDL mixed with 500 l of cholesterol reagent, which had been diluted with

distilled water (ratio 4+1), then incubated for 10 minutes at room temperature, centrifuged at 4000 rpm for 10 minutes, the resulting supernatant solution (Prangdimurti et al., 2007).

First, the blank solution (1 ml HDL cholesterol reagent) was measured, then the standard solution (1 ml HDL cholesterol reagent + 100 l supernatant solution) was measured. Then 100 l of serum was taken and mixed with 1 ml of HDL cholesterol reagent, then put into a test tube and mixed until homogeneous, incubated at room temperature for 20 minutes, and then measured (Prangdimurti et al., 2007). Measurements using a micro lab 300 spectrophotometers with a wavelength of 546 nm, the results were recorded.

### Calculation of LDL Levels The

The most widely used technique by clinical laboratories to measure patients' LDL levels is by using the Friedewald formula as follows:

$LDL\ level = Total\ cholesterol - HDL - TG/5$  It is assumed that TG/5 is the level of VLDL (Prangdimurti, et al, 2007 ).

### Data Analysis

Observational data were analyzed statistically using ANOVA (*Analysis of Variance*). This statistical analysis uses SPSS (*Statistical Product and Service Solution*) version 16.

## RESULTS AND DISCUSSION

Melting point determination was carried out using a *melting point apparatus*, and melting point data is shown in table 2 below.

**Table 2.** Melting point data

Sample /	Melting Point (°C)				
	time 0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
Beef fat	37	42.1	43.3	43.3	43.3

Based on the data in Table 2, it can be seen that the reaction chemical interesterification has run perfectly after the reaction has been running for 60 minutes. The addition of interesterification time no longer affects the change in melting point. This short time is due to using a Na-methoxide catalyst which can speed up the

reaction, so it does not take long to reach equilibrium. Interesterification has run perfectly if the melting point of fat does not change anymore (Silalahi, 2006). Chemical interesterification results in randomization of the acyl groups in the triglycerides Interesterification can occur without using a catalyst but requires a very high

temperature., achievement of equilibrium (equilibrium) is very slow. The triglycerides will decompose, polymerize and produce a lot of free fatty acids. The temperature required for interesterification without a catalyst reaches 300oC or even higher. For this reason, a catalyst is used that can speed up the reaction and lower the temperature (Silalahi, 1999; O'Brien, 1998).

### Lipid Profile After Giving Beef Fat The

profile of total cholesterol, triglycerides, HDL, and LDL after giving beef fat with variations in interesterification time in guinea pigs is shown in table 3 and Figure 1 below:

**Table 3.** Average levels of lipid profile on reaction time

Samples	Reaction (minutes)	Average Level (n=3) mg/dl			
		Cholesterol	Triglycerides	HDL	LDL
Beef Fat	0	62.27 ± 16.02	62.87 ± 14.08	11.80 ± 2.15	37.84 ± 13.41
	30	63.93 ± 11, 76	64.53 ± 10.95	15.67 ± 1.63	35.36 ± 9.82
	60	68.67 ± 19.65	69.20 ± 20.24	14.33 ± 3.01	40.43 ± 15, 18
	90	68.67 ± 19.65	69.27 ± 20.98	15.26 ± 2.60	39.55 ± 15.72
	120	69.07 ± 18.71	69.67 ± 20.09	13.60 ± 1.05	41.53 ± 14.94

Note: significantly different (ANOVA, P < 0.05) compared with reaction times of 0 minutes (a), 30 minutes (b) and 60 minutes (c).

After giving interesterified beef fat, total cholesterol, triglycerides, and LDL increased more than before interesterification. According to Silalahi and Nurbaya (2011), palmitic acid in beef fat is in the sn-1 and sn-3 positions, with such a position that palmitic acid is not absorbed, so it is not atherogenic. Berry (2009) stated that palmitic acid in beef fat without interesterification was at positions sn-1 and 3, not increasing total cholesterol, triglycerides, and LDL. However, research on giving beef fat without interesterification gave different results. There was an increase in cholesterol, total triglycerides, and LDL due to the cholesterol content in beef fat, which can trigger the formation of cholesterol in the guinea pig's body. According to Silalahi (2006), cholesterol content is found in animal fat.

The increase in total cholesterol, triglycerides, and LDL in interesterified beef fat occurred due to the displacement of palmitic acid from the sn-1 and sn-3 positions to the sn-2 position to increase potential atherogenicity. According to Silalahi (2006), the interesterification reaction will change the atherogenicity of the fat due to the displacement of the position of the fatty

acids in the molecule, especially palmitic acid. The initial stage of atherosclerosis is caused by high LDL levels in the circulation. This LDL can be trapped in the intima and will undergo oxidation. This oxidation event will stimulate the cell surface to attract monocytes into the intima. In intima, monocytes will turn into macrophages which will eat oxidized LDL. The more LDL that is eaten causes the macrophages to be packed so that the macrophages will be shaped like foam and then will clog blood vessels (Silalahi. 2006). From total cholesterol, triglycerides, LDL on days 1 and 7 in beef fat, the results increased and then decreased the next day, while without a tendency to continue to increase, there is no theoretical basis for this, but the author, after seeing the behavior of experimental animals (guinea pigs) that after doing so has formed a fat that can make guinea pigs feel full and no appetite.

Increased LDL lipid profile in beef fat indicates an increase in both fats. According to Hermansen et al. (2003), the ratio of LDL: HDL is the strongest predictor of coronary heart disease. The LDL: HDL ratio is described in these findings. According to Fernandez (2001), guinea pig



cholesterol metabolism is the same as cholesterol metabolism in humans, including the ratio of LDL: HDL.

The LDL: HDL ratio after giving interesterified beef fat is shown in Table. The LDL: HDL ratio after giving interesterified beef fat can be seen in Table 4. It can be seen that after giving

interesterified beef fat on day 1 to day 21 does not indicate the occurrence of atherosclerosis where all data on the LDL: HDL ratio were not high (<5), but the LDL: HDL ratio on the seventh day of 4.23 was approaching 5.

**Table 4.** LDL: HDL ratio after administration of interesterified beef fat

Day to -	Average Level (n=3) mg/dl		LDL: HDL
	HDL	LDL	
0	14.67	20.27	1.38
1	14.00	52.53	3.75
7	14.33	60.60	4.23
14	14,00	35,67	2,55
21	14,67	33,06	2,25

## CONCLUSION

The effect of interesterification of beef fat shows an increase in atherogenicity. The comparison between interesterified and uninterested beef fat was seen from the increase in total cholesterol, triglycerides, LDL and decreased HDL levels after interesterification, respectively  $69.20 \pm 20.24$ ;  $68.60 \pm 18.81$ ;  $40.43 \pm 15.18$ ;  $14.33 \pm 3.01$  mg/dl compared with no interesterification  $62.27 \pm 16.02$ ;  $62.87 \pm 14.08$ ;  $37.84 \pm 13.41$ ;  $11.80 \pm 2.15$  mg/dl (ANOVA,  $P < 0.05$ ).

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