Profile of Decreasing Fat and Cholesterol in Chicken Meat Using Lipase Enzyme from Coconut Haustorium

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Abstract. Thigh chicken meat is one of high fat contained foods (7.2%) compared to beef (6.8%) and lamb meat (4.9-5.2%) with high consumption levels. Consumption of high fat contained foods can cause cardiovascular diseases such as hypertension, coronary heart disease, heart failure, and stroke. Cardiovascular diseases causing 17.9 million people die or 31% from global deaths in 2016. The crude lipase enzyme extracted from coconut haustorium can efficiently hydrolyze chicken meat fat into free fatty acids and glycerol. This research was started by reducing the size of coconut haustorium, then mixed with chicken meat and buffers with formulation 1:10:40. After that, sample is reacted in water bath shaker with 200 rpm of agitation, (30, 40, 50, and 60)°C temperature, and (3, 6, 9, and 12) hours reaction time. The yield of this study was the highest reduction in chicken fat is treatment of 50°C for 6 hours that can decrease 60% of chicken fat. The highest cholesterol reduction was at a temperature of 60°C for 12 hours that can decrease 97% cholesterol.

1. Introduction
Chicken meat is consumed by many people as a source of animal protein. According [7], every 100 grams of chicken meat contains 18.2% protein, 25% fat, and 404 Kcal calories. Average consumption of chicken meat race (broiler) is 4,797 kg in 2015 and increased to 5,110 kg in 2016 [4]. The average consumption of chicken meat per capita during the week in 2017 was 0.124 [5]. Thigh meat contains 7.2% of fat, higher than 6.8% fat from beef, and 5.9-5.2% in sheep muscle parts [6]. Fat-rich food by itself will affect blood cholesterol levels which are risk factors for cardiovascular disease [3].

Cardiovascular disease is the leading cause of death in various developed countries and tends to increase as a cause of death in various developing countries [11]. Cardiovascular diseases such as hypertension, coronary heart disease, heart failure, and stroke cause 17.9 million people die or 31% of all global deaths [30]. Data from [13] shows the prevalence of hypertension in Indonesia based on measurement to age 18 years old is 25.8% with health personnel coverage only 36.8% and 63.2% cases in the community not diagnosed with prevalence increasing with increasing age from respondent.

One alternative to reducing chicken fat and cholesterol is enzymatic reaction using lipase enzyme. Lipase enzymes hydrolyze triglycerides, diglycerides, and monoglycerides into free fatty acids and glycerol [21]. Crude lipase extract can efficiently hydrolyze chicken meat fat under acidic conditions [1]. Lipase enzymes from coconut haustorium have activity of 0.0581 U/ mg, waste watermelon and
orange peels provide lipase activity of 1.36 U/mL, rice bran of 0.040 U, and walnut seeds of 0.03 - 0.04 µmol/mg [26], [29], [9], [8].

Coconut haustorium contains lipase enzymes because coconut store their food in coconut meat in the form of fat then converted into cellulose as a constituent of coconut shoots. The advantages of lipase enzymes from coconut haustorium are active at room temperature and naturally immobilized. Enzymes immobilized in certain matrices can also produce enzyme stability and prevent contamination between products and enzymes [19].

There are several factors that can affect the enzyme activity from coconut haustorium, such as temperature and reaction time. Therefore, this study was conducted to determine the optimum temperature and duration of reaction in reducing fat and cholesterol levels of chicken meat using lipase enzyme from coconut haustorium.

2. Methodology
The main raw materials used in this study are coconut kentos and chicken meat. The main tools used are waterbath shaker, spectrophotometer, and soxhlet. This research was conducted by preparing small and mild chopped coconut haustorium, chicken meat fillet, and buffer solution. The ingredients mixed with formulation coconut haustorium: chicken meat: buffer is 1: 10: 40. After that, it is reacted in a waterbath shaker with agitation of 200 rpm. Temperature variations used are (30, 40, 50, and 60)°C and reaction time (0, 3, 6, 9, and 12) hours. Test parameter of chicken meat before and after reaction were fat and cholesterol content. While test parameter of suspension fluid before and after reaction were the levels of Free Fatty Acid (FFA) contained in it.

3. Results and Discussion
In chicken meat, the results of enzymatic reactions were analyzed by reducing fat and cholesterol levels. Fat or lipids are organic compounds that are composed of carbon and hydrogen and are not soluble in water but are soluble in nonpolar solvents. Fat provides essential fatty acids such as linoleic and alpha-linolenic acids, provides lipophilic vitamins (A, D, E, and K), the main energy source for the body, creates a feeling of satiety because it effects slowing gastric emptying, reducing carbohydrate bioavailability, and increasing taste, aroma, and texture of food [17]. The results of testing the fat content of chicken meat at the reaction temperature variations of 30, 40, 50, and 60°C during the 6 hour reaction showed a decrease in chicken fat which can be seen in Figure 01.

![Figure 1. Relation of enzymatic reactions temperature to chicken meat fat levels](image-url)

The results showed decrease of fat from control sample at various temperature variations for 6 hours of enzymatic reaction. The control sample had 5% fat contents and based on the treatment of 30°C, 40°C, 50°C, and 60°C temperature, the samples fat content in sequence are 4%, 3%, 2%, and 3%. Treatment of 50°C for 6 hours can reduce fat by 60%. The results of the study of [13] showed that...
the lipase from coconut kentos which was dried for 30 days had an optimum temperature of 60°C and the optimum reaction time was 90 minutes. The results of the study of [2] showed that the lipase enzyme from rubber seed sprouts had an optimal temperature of 40°C and its activity decreased at 50°C. The different optimal temperature of lipase enzyme is affected by enzyme are protein so that even though enzyme activity increases with increasing temperature, but if the cofactor is not added, enzyme stability decrease because of denaturation. In addition, the formation of free fatty acids as a result of fat hydrolysis can become an inhibitor in enzymatic reactions so that the reaction becomes saturated and enzyme activity decreases at higher temperatures in the same reaction.

The recommendation of maximum total fat consumption per day is 30% of total energy, which includes 10% saturated fatty acids (SFA), 10% single unsaturated fatty acids (MUFA) and 10% plural unsaturated fatty acids (PUFA) [14]. The main composition of broiler meat is saturated fatty acids, i.e fatty acids that not have double bonds [23]. Consumption of high saturated fatty acid caused liver produce LDL cholesterol in large quantities that can cause heart disease and increase blood cholesterol levels [18].

Cholesterol is a complex fat compound in every cell of the body [20]. Cholesterol serves as the initial material for the formation of bile, cell walls, vitamins and certain hormones, such as sex hormones and others [10]. 80% of cholesterol is synthesized by liver and 20% goes with food ingredients [25]. Cholesterol derived from food plays an important role, because it is the main sterol in the body and the components of the cell surface and intracellular membrane [24]. However, consumption of foods that are high in fat and cholesterol will increase total cholesterol levels and LDL levels so that the liver will have enough cholesterol levels and stop taking LDL which can increase total cholesterol levels [22]. Cholesterol test results of chicken meat at temperature reaction variations of 30, 40, 50, and 60°C for reaction times 0, 3, 6, 9, and 12 hours showed decreased chicken meat cholesterol as can be seen in figure 2.

![Figure 2](image_url)

**Figure 2.** Effect of temperature and reaction time on changes in chicken meat cholesterol levels

The results showed decrease of cholesterol from the control sample at various variations of temperature and reaction time. The control sample had absorbance on a spectrophotometer of 1.129. The most decrease in absorbance of cholesterol was at a temperature of 60°C with absorbance value of 3 hours, 6 hours, 9 hours, and 12 hours reaction sequentially as follows: 0.426; 0.160; 0.074; 0.035. The results of this study were temperature 60°C for 6 hours efficiently reduce cholesterol by 85% and after 12 hours it decreased by 97%. The optimal temperature difference in decreasing fat and cholesterol in chicken meat is caused by difference enzymes that hydrolise ester bonds in fat and
cholesterol. In the hydrolysis of cholesterol there is a cholesterol esterase enzyme that breaks down cholesterol into sterols and free fatty acids.

The results of the study by [24] shows that cholesterol level in chickens was 142.22-160.00 mg/dl with HDL levels of 36.47-47.38 mg/dl and LDL of 99.80-119.29 mg/dl. [15] describes normal blood cholesterol levels between 125-200 mg/dl. Miruka in [16] shows HDL levels of normal broiler 40-60mg/dl and normal chicken LDL levels of 95-125 mg/dl. [31] determine that excessive cholesterol levels in the blood will easily stick to the inner wall of blood vessels. Excess LDL through the oxidation process will form clots so that there is a narrowing of the blood vessels. Every 4 (four) ounces of beef or chicken meat contains 100 mg of cholesterol which contains saturated fatty acids that can increase levels of K-LDL (LDL cholesterol).

Free fatty acid is analyzed from enzymatic reaction liquid. The amount of free fatty acids is the result of the hydrolysis of chicken meat’s fat and cholesterol. Free fatty acid is a substrate obtained as a result of breaking the ester bonds in fat and cholesterol of chicken meat. Fat metabolism begins with the hydrolysis process of fat (triglycerides) from food by the lipase enzyme to produce free fatty acids and glycerol. The results of testing free fatty acids on the enzymatic reaction based on reaction temperature variations of 30, 40, 50, and 60⁰C for the reaction time of 0, 3, 6, 9, and 12 hours showed an increase which can be seen in figure 3.

![Figure 3. Effect of temperature and reaction time on changes in free fatty acids](image)

The results showed increased of free fatty acids from control sample to various temperature and reaction times. Free fatty acids before the enzymatic reaction is 1.79% and increase of up to 6.59% after a reaction time of 12 hours at 40⁰C. The difference rate of free fatty acids formation is influenced by the type of fatty acids bonded to hydrolyzed fat or cholesterol. In general, chicken meat contains long chain fatty acids with chain lengths C14 to C20. [12] showed fat from chicken meat containing myristic acid (C14: 0) 0.74%; palmitic acid (C16: 0) 27.24%; linoleic acid (C18: 2) 16.36%; oleic acid (C18: 1) 38.35% and stearic acid (C18: 0) 5.56%. Long chain fatty acids released from enzymatic hydrolysis will be further hydrolyzed into simpler fatty acids such as butyrate and acetate. This process increase the amount of free fatty acids formed in the liquid resulting from enzymatic reactions. The level of hydrolysis of cholesterol esters from different fatty acids is in the following order: linoleic> oleate> stearic> palmitate> capriate> caprylate> myristate> lauric, caprate> caproat> butyrate, acetate [28].
4. Conclusion
From several treatments of temperature and reaction time, it was found that the highest reduction in cholesterol levels was obtained at 60°C for 6 hours that decreased by 85% and after 12 hours decreased by 97%. While the treatment temperature of 50°C for 6 hours can reduce fat by 60%.

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