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Effect of Variation Time Storage Bengkoang (Pachyrrhizuserozus) to Cholesterol and Lypase Activities *Rattus norvegicus*

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ABSTRACT - Inulin is a fructose polymer that is difficult to process with human digestion but can be fermented by Bifidobacteriabecome Short Chain Fattic Acids (SCFAs) like lactic acid, propionate and butirate. It is used for improving intestinal villi work and decreasing bad cholesterol levels. Yam (Pachyrrhizuserozus) is a leguminous plant from tropical America that is rich in inulin. The aim of this research is to get to know the relationship between the variations in the time storage of yam tubers to cholesterol levels and duodenum lipase activity. This research used The Post Test Only Control Group. The experimental animals used were *Rattus Norvegicus*. The groups are: a control group (K) with regular food and others using food mixed with yam with variations of (1 day (P1), 14 days (P2), and 28 days (P3)). After 50 days, duodenal and blood were taken to know the activity of the lipase duodenum and cholesterol levels. The result of this research are: 1. Blood cholesterol levels K, P1, P2, and P3 were 68.40, 51.80, 67, 81.80 (mg / dL). Lipase activity K, P1, P2, and P3 was 0:07, 0:05, 0:11, 0:13 (IU / mL). There is a relationship between the variations in the time of storing the tuber and the animal lipase, and blood cholesterol levels.
Keywords: Yam, Lipase, Blood Cholesterol

INTRODUCTION

Bengkoang/Yam are legumes that have tubers. They come from America and are then grown on a large scale in Indonesia. The species that can live in Indonesia is *Pachyrhizus erosus* [1]. Indonesians use these tubers in cosmetic materials and food because of their nutritional quality. The tubers, which have yellow skin and are white inside, contains 80-90% water [2]. *Pachyrhizus erosus* contains water, starch, vitamin C, B1 and minerals such as Ca, P and K. Other content includes a dietary fibre called inulin. Inulin is useful to improve blood parameters and the human digestive workings.

Inulin contains 35 units of fructose with a β-2,1glycoside bond with a non-reducing glucose terminal. This dietary fibre is useful to decrease blood glucose and cholesterol levels. In the large intestine, inulin produces short-chain fatty acids (SCFA) like L-lactate by Bifidobacteria that have been demonstrated *in vitro* and *in vivo* [3]. The regulation of lipids, starting from SCFA, is then converted into propionate. Propionate is absorbed into the portal vein is then metabolised in the liver. SCFA is involved in the regulation of body lipids and is used in HMG-CoA reductase inhibitors for inhibiting the catalysed formation of mevalonic acid. Mevalonic acid is a precursor of cholesterol. so it is not able to form excess cholesterol in the blood to then be distributed [4]. Inulin is one of the dietary fibres that is soluble in water. It can't be digested by digestive enzymes. The hygrocospic effect of inulin can bring it in to the large intestine without it changing. Another effect of it is that it holds water easily, so it makes a viscous gel which stops the activity of enzim. In Yuanita's research (2010), that dietary fibre has a negative impact on digestive metabolism in enlarging the volume and being able to bind protein digestive enzymes, so the enzyme activity decreases. However, the mechanism of binding by dietary fibres is not the same as by inhibitors. Fibres only interact with the enzyme while the enzyme remains active, but the activity is decreases [5].

Based on Anggriawan's research, there is 1.9% inulin in an extraction of 100 grams bengkoang (after harvest) tubers with 50% ethanol. It is higher than in 500 grams of dahlia bulb, where there is only 0.9% of the inulin within the same concentration of ethanol. But storage on the market for a long time at an unspecified temperature can affect the inulin content of the tubers. Inulin can be depolymerised by microorganisms in the air [6]. In the process of storing fruits, tubers and vegetables for a long time can change the physical and chemical properties inside. This is because after harvesting the vegetables, tubers and fruits are also still in a process of respiration [7]. According to Pantastico (1989),

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respiration can be divided into three levels: 1)Polysaccharides change into simple sugars; 2) Oxidation of sugars into pyruvic acid; 3) The transformation of pyruvate and other organic acids aerobically into CO2, water and energy. The effect of these enzyme microorganism products changes the levels of fibre and complex carbohydrates in the tubers.

Research Methods

Tools

This study used several tools designed for the manufacture of feed and the maintenance of animals namely digital balances, label paper, filtering tools, knives, blenders, ovens, plastic containers, trays, animal experimental cages, feed containers, cameras, and drinking bottles.

The tools used for blood glucose and enzyme amylase trials are 2cc, microlab-200, blood tubes, pipettes and micropipettes, UV-Vis spectrophotometers, pH meters, centrifuges, trophies, test tubes, centrifugation tubes, cuvads, Vortex appliance, water bath, measuring cup, analytical balance, aluminium foil, vacuum filter, thermometer, waterbath, spindle, stirring glass, watch glass, funnel, and vials.

Material

The materials used in this study were *bengkuang* samples which varied in storage time, wheat flour, anchovy, palm oil, vitamins (premix), cornstarch, skim milk flour, and bone meal. This is well as the blood of mice taken from the heart, CHOD-PAP reagents (buffer phosphate 6,5, phenol, 4-aminoantipirin, cholesterol esterase, cholesterol oxidase, peroxidase), HCL buffer, alcoholperoxidase, 0,1 M sodium phosphate buffer for pH 6 and 7, substrate (60% (v / v), olive oil in n-hexane, aquadest, n-hexane, 5% (w / v) cupric acetate pyridine pH 6,0, and oleic acid.

RESEARCH PROCEDURE

Sample Preparation

For the first step, old-treated *bengkuang* peeled and washed and then smoothed using a blender. The result of the smoothing process was then weighed in to 172 grams for each portion and filtered to separate the filtrate from the dregs. After that, the filtrates were stored in the freezer and melted before being administered to the animals with a.d bilitum every day @ 20 mL, while the dregs were mixed with standard feeding food.

For the next step, all of the experimental animals used were given standard feed for 2 weeks, then the animals were divided into 4 groups. Each group had the same weight. 3 groups were fed standard feeds added to the treated *bengkuang* at 1, 14 and 48 days, and the other group were fed as controls. After that, the feed was stored and labelled.

Determination of Blood Cholesterol Level (BBLK Surabaya 2013)

The animal was tested after surgery for ± 50 days, and blood drawn through the heart. Blood serum was taken as 10µL then CHOD-PAP 1mL was added as a reagent, and vortex. This was left to stand for 10 minutes. After 10 minutes of activation, the serum activity was measured using a microlab 200 at a 505nm wavelength. However, the blanks and standards must be measured first before measuring the sample. Blanko was made using aquadest while the control used a standard cholesterol solution instead of a blood serum solution. Finally in the process of measuring the activity, the levels of glucose in the blood serum of the mice was tested.

Making Duodenum Extract (Megiandari, 2009)

The animals were dissected after ± 50 days of treatment for their duodenum. The intestines of the experimental animals were cut after they were washed with aquadest and parts of the intestine were identified. Then the intestines wre cut according to the duodenum to look inside. The inside of the duodenal intestine was then scraped carefully to obtain the upper layer of the intestine (mucosa). After that, the upper layer of the intestine (mucosa) (2% v / v) was dissolved in a 0.05 M phosphate buffer pH 7.0 then stirred with vortex for one minute. The duodenal mucosal fluid was then separated between the supernatant and sediment using a centrifuge at a speed of 6000 G with a temperature of 4 ° C. for 10 minutes. The supernatant obtained was an enzyme extract used for the amylase and lipase enzyme activity test.

Determination of Absorbance of Oleic Acid Solution (Yuliani, 2007)

The standard oleic acid curve was made with some variations in the concentration of the oleic acid. The required concentrations were 3.5; 7; 10.5; 14; And 17.5 (x 10-4 M). The variation in the concentration

of the solution was made by using a standard solution of oleic acid 0,007 M. Then the solution was taken by as much as 0.5; 1; 1.5; 2; 2.5 mL. After that, it was diluted with n-hexane to 10 mL. The solution was added to 1.0 mL isooktan and 800 µL 5% (w / v) coupry pyridine coupry pH 6.0.

Th mixture was then silenced for 10 minutes with the aim of developing the colour. The solution was then measured with a spectrophotometer at 707 nm to determine its absorbance.

Lipase Enzyme Determination (Yuliani, 2007)
The sample measurements were made using natural substrates (olive oil) in accordance with the method suggested by Marseno et al. (1998). The hydrolytic lipase activity test was carried out in a reaction mixture of 1.0 mL 0.1 M sodium phosphate buffer for pH 6, and 7, 1.0 mL enzyme solution, and 4 mL substrate (60% (v / v) olive oil in isooktan). The mixture was then emulsified with the vortex until homogeneous. The final buffer concentration in the polar phase was 0.1 M. Then the mixture was incubated at 30 ° C. for 20 minutes with a wobble of 150 wobbles per minute.

Activity	Cholesterol level mg/dL	Score F,p
K (regularly food)	68.40[bcd]	F= 7.280 P= 0.03
P1 (with bengkoang that saved in 1 day)	51.80 [acd]	
P2 (with bengkoang that saved in 14 day)	67.00 [abd]	
P3 (with bengkoang that saved in 28 day)	81.80 [abc]	

The reaction was stopped after 20 minutes with 150 wobbles per minute, and then incubated on ice for 5 minutes. For this test, the control was done by replacing the enzyme solution with aquadest.

The fatty acid liberated in the reaction was measured by taking 3.0 mL of the non-polar phase and feeding it into the test tube, then adding 1.0 mL isooktan and 800 µL 5% (w / v) of pyridine chitri pyridine pH 6.0. After being silenced for 10 minutes to develop the colour, the absorbance was measured with a spectrofoto-meter at 707 nm. The fatty acid content present in the sample was then determined by a standard curve made through oleic acid as it is a free fatty acid. One unit (U) of hydrolytic lipase activity is defined as an enzyme activity in relation to it producing 1 µmol of product (liberated fatty acid) per minute. Lipase enzyme activity can be calculated using the following formula:

$$\text{Enzime Activaty}\left(\frac{u}{mL}\right) = \frac{[\text{Asam Oleat}]}{\text{Mr}} \text{ x Fpx} \frac{\text{Venzim}}{t}$$

DISCUSSION
This research used *bengkuang* from Bojonegoro and took into account the variation of storage of 0, 14, and 28 days. The experimental animal samples used came from the Biochemistry Laboratory of the Medicine Faculty of Airlangga University Surabaya and had been fed variations of yam that has been adapted to regular food over 50 days. The blood and duodenum was examined for blood glucose, total cholesterol and the activity of the lipase enzyme. The results and discussion in relation to each test has been described as:

Determination of Blood Cholesterol Level
In this study, the effect of the prolonged storage of *bengkuang* against blood cholesterol was obtained based on the amount of cholesterol in mL serum blood. The blood cholesterol level was read by way of the enzymatic cholesteroloxidase (CHOD-PAP) method. Principally, this test used the following equation:

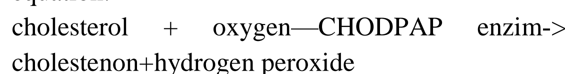
cholesterol + oxygen—CHODPAP enzim-> cholestenon+hydrogen peroxide

Table 1.1 Blood Cholesterol
There is a decrease in the cholesterol in P1 to K caused by the presence of high inulin-soluble dietary fibre in order to regulate cholesterol levels in the blood. Fibre can affect the absorption of fat by binding the fatty acids, cholesterol and bile salts in the gastrointestinal tract, and so decreases the cholesterol. Fatty acids and fibre-bound cholesterol results in the absence of much needed micelle in the absorption of fat through the unstirred water layer in the enterocytes.

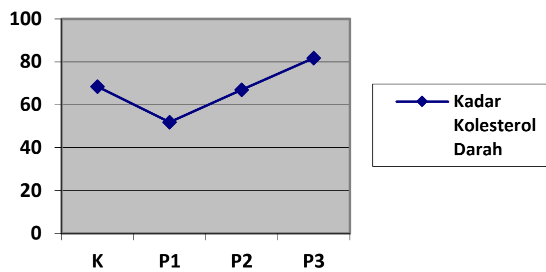

Figure 1.1 Blood Cholesterol

As a result, the fat that cannotbe absorbed will go directly to the colon to be excreted through faeces or degraded by intestinal bacteria. The increased fatty acid excretion, cholesterol and bile salts through the faeces and bile salts undergoing enterohepatic cycle are also reduced. Bile salts and lost cholesterol will increase the use of cholesterol present in the blood for new bile salt systesis, thereby lowering blood cholesterol overall. In addition to these mechanisms, there is a change in the pattern of bile salts from colic acid to chenodeoxycholic acid which inhibits the 3-hydroxy 3-methylglutaryl (HMG) CoA reductase needed in cholesterol synthesis.

According to Tala (2008), studies in animals have been able to prove that propionate or other short-chain fatty acids formed will inhibit fatty acid synthesis. There was an increased total blood cholesterol level in the treatment 28 day *bengkuang* storage. This increase occurs due to the time of some storage constraints that can damage the inulin structure. Inulin in the degraded yam during storage can produce excessive fructose in the body. From a study conducted by Moore, the mixing of 7.5 grams of fructose in 75 grams of standard glucose for oral glucose tolerance test showed a decrease in the glycemic response. In the administration of fructose that is too high in the blood, it causes the process of glycation, which is the process of reacting fructose and glucose with the body tissues that cause diabetes complications (Journal of diabetes, 2000).

Activity	Kadar Lipase mg/dL	Nilai F,p
K (regularly food)	0.07^{bcd}	F= 42.148 P= 0.000
P1 (with bengkoang that saved in 1 day)	0.05 ^{acd}	
P2 (with bengkoang that saved in 14 day)	0.11 ^{abd}	
P3 (with bengkoang that saved in 28 day)	0.13 ^{abc}	

Another study of the negative effects of high-fructose feeding was also performed on mice for 10 weeks. The results showed the production of new fat cell cells in the heart, liver and other digestive organs (Medical news, 2010). The excessive consumption of fructose is also thought to play a role in insulin resistance to obesity, cholesterol and triglyceride disorders that will cause a person to have metabolic syndromes (Elliot, 2002). In Peter's study in 1985, they described increases in triglyceride levels that can increase the total cholesterol levels in the blood. The evidence from several previous studies above reinforces that the results obtained in this study that the treatment of feeding variation over 28 days old *bengkuang* occurrence increased the total blood cholesterol levels by 19.5%.

Determination of Lipase Enzyme

In this study, the effect of *bengkuang* storage on lipase enzyme activity was obtained based on the amount of oleic acid formed. The oleic acid formed comes from the degradation of olive oil which can be detected by the concentration results of the reaction with the chitridine acetate reagent. The reaction of fatty acids with the acrylic copper pyridine reagent formed a copper complex that produces a blue colour. The data obtained in this study was in the form of absorbance converted into an oleic acid concentration, after which the calculated lipase enzyme activity was analysed with the formula. The activity of the lipase enzyme (Appendix 6) was then taken by average and made in to a graph in Table 4.4 and Figure 4.5.

Table 1.2 Lypase activity

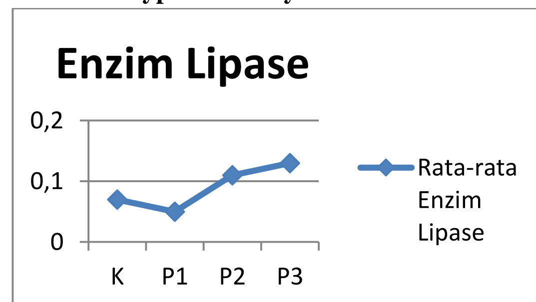

Figure 1.2 Lypase activity

The curve shows a decrease in lipase enzyme activity resulting from the ability of the fibre to retain water in its matrix which causes the ability of the food to interfere with digestive enzymes to . The viscous gel formed can affect the enzymatic hydrolysis process in the gastrointestinal tract. Inulin, which is a soluble fibre can inhibit the work of the lipase enzyme that inhibits the digestion of fat.

CONCLUSION

The results of the research are:

There is an influence of yam feed on the variations in storage duration on the amylase doudenum enzyme activity of *Rattus norvegicus*. Based on long tube storage study effect on enzyme activity approach using P2 standard feed of 0.07 U / mL. The activity of the standard feed amylase enzyme was 0.08 U / mL

There is an effect of yam feed on the variations in storage duration in *Rattus norvegicu* lipase doudenum enzyme activity. Based on an old tuber storage study, the effects on lipase enzyme activity for enzyme activity was approached using P1 standard feed of 0.05 U / mL. Standard lipase feed enzyme activity was 0.07 U / mL

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