In vivo study of the hypocholesterolemic effect of bread made from flour, starch, and fiber-rich flour from purple sweet potato on rats

Michael Rapagha Sembiring[1*], *Elisa* Julianti[1] , and *Linda Masniary* Lubis[1]

[1]Departement of Food Science and Technology, Universitas Sumatera Utara, Jl. Prof. A. Sofyan No. 3, Medan , 20155, North Sumatera, Indonesia.

Abstract. This research was purposely to study and determine the physicochemical characteristics, sensory and hypocholesterolemic effects of purple sweet potato bread made from flour, starch, and fiber-rich flour from purple sweet potato starch processing waste on white male species Wistar rats conducted in vivo. This study used a non-factorial randomized block design model with the treatment of 100% standard feed, 25% mixture of purple sweet potato bread, and a combination of 50% purple sweet potato bread to healthy rats, and treatment of 100% standard feed and gemfibrozil, 25 % mixture of purple sweet potato bread, and a mixture of 50% purple sweet potato bread against hyper cholesterol rats. This research result shows the different hypocholesterolemic effects on the percent decrease in total cholesterol, triglycerides, LDL (Low-Density Lipoprotein), and an increase in HDL (High-Density Lipoprotein) in the [1]blood serum of rats as test animals.

1 Introduction

Bread is one of the processed foods widely known by the world community as one of the daily consumption foods. Purple sweet potato (PSP) is one type of sweet potato that can be used as a functional food because of its high anthocyanin content, attractive color and it can be used to prevent various types of degenerative diseases(1). Purple sweet potato can be processed into nutrient-rich flour, starch, and also fiber-rich flour. PSP starch contains oligosaccharides that can provide a longer full effect when consumed, while PSP fiber has a higher dietary fiber content which contributes to delivering a hypocholesterolemic product. Purple sweet potato has antioxidant activity from anthocyanin compounds that have anti-diabetic abilities such as lowering blood sugar, inhibiting the production of free radicals, increasing insulin secretion, preventing insulin resistance, and inhibiting the activity of the maltase enzyme that produces glucose(2). Sweet potatoes are also rich in carbohydrates in the form of starch, contain high fiber, various types of minerals, and vitamins, especially vitamin A, as well as bioactive components in the form of anthocyanin color pigments, which also have antioxidant activity.

* Corresponding author: elizayulianti@yahoo.com

2 Material and Method

Six-month-old purple sweet potatoes are fresh harvested from farmers in the Pakpak Bharat, North Sumatera, Indonesia. Sodium metabisulfite is as chemical additive in the flour production process. Bread ingredients include instant yeast, salt, shortening, sugar, skim milk, bread improver, xanthan gum, and water. Chemical reagents in the chemical examination of purple sweet potato bread and rat treatment ingredients such as standard feed, quail eggs, gemfibrozil, and propyl thiouracil.

The equipment used to manufacture of flour, starch, and fiber are knives, buckets, hammer mill, filter cloth, drying oven, scales, sieve shaker, 80 mesh sieve, 60 mesh sieve , and baking sheet. The equipment used for bread processing is a mixer, bread maker, oven. The equipment used to characterize the chemical of food products is analytical balance, centrifuge, aluminum dish, porcelain dish, Soxhlet, hot plate, Kjedhal flask, Rapid Visco Analyzer, texture analyzer, spectrophotometer, and other glassware. In vivo treatment of rats was used in isolation cages, squits, etc.

2.1 PSP flour, starch, and Fiber-rich flour process

2.1.1 PSP Flour Process

Purple sweet potatoes were sorted by size, shape , and color. Then PSP was washed, peeled ,and sliced with the thickness of ± 2 mm using a slicer machine to obtain sweet potato chips. Sweet potato chips were then wrapped a filter cloth then soaked in 2000 ppm sodium metabisulfite solution for 15 minutes then drained, washed with running water. The PSP chips are then dried using an oven at 55ºC for 12 hours until the chips become dry, which is indicated by a rustling sound when the flakes are kneaded by hand. The dried flakes were ground using a disc mill, and sieved with an 80 mesh sieve size. The PSP flour is packaged in polyethylene plastic packaging before use.

2.1.2 PSP Starch and Fiber-rich Flour Process

Purple sweet potatoes were washed, peeled and shredded using a grating machine and soaked in 2000 ppm sodium metabisulfite solution with a ratio of 1:3 (w/v). Then squeezed and filtered, the PSP pulp then dried in an oven at 60ºC, and the dry fibers were ground using a hammer mill, sieved with a 60 mesh sieve size to obtain PSP fiber-rich flour. The filtrate was deposited for 3 hours so that the liquid part (supernatant) and starch residue were obtained. The starch was then dried in an oven at 50ºC for 12 hours. The dry starch was ground using a disc mill and sieved through an 80 mesh sieve size.

2.2 Preparation of PSP bread

Bread production refers to the bread-making procedure according to (3) with several modifications, which seen in Table 1. PSP flour, PSP starch , and PSP fiber in a 75 : 5 : 20 mixed with other dry ingredients such as sugar, yeast, skimmed milk, salt, and bread improver mixed using a mixer with low speed, then while mixing add eggs and water gradually until the dough forms. After the dough is forms, add shortening as the mixer is still on till the dough is dull.

The resulting dough was then proofed at room temperature for 30 minutes then pressed to release the gas. Place the dough in a mold and proof for 1 hour. After 1 hours of proofing, the dough was baked at 190°C for 30 minutes. The bread were cooled for 30

minutes at room temperature on a baking shelf before sealing and packing in polyethylene plastic.

2.3 In vivo hypocholesterolemic effect of PSP bread on male white of Wistar rats

30 of 2-3 months healthy male of white Wistar rats with the average body weight of 150-200 gram were acclimatized for one week. Then the rats were divided into six groups, each consisting of 5 rats. Three groups of rats were given a basal diet and grouped as healthy rats, in comparison, the other three groups were given a high-cholesterol diet in the form of quail egg yolk and 0.01% propylthiouracil for five weeks so that they experienced hypercholesterolemia. All test animals were then measured their blood cholesterol total, LDL and HDL cholesterol, and triglyceride and recorded as pretest levels. Blood is drawn from the lateral vena of the tail surface. Each group of test animals was treated as :

A1 : Healthy rats and fed with 100% standard fed (Negative control)
A2 : Healthy rats and fed with 25% PSP bread mixture with standard fed
A3 : Healthy rats and fed with 50% PSP bread mixture with standard fed
A4 : Hypercholesterolemic rats and fed with 25% PSP bread mixture with standard fed
A5 : Hypercholesterolemic rats and fed with 50% PSP bread mixture with standard fed
A6 : Hypercholesterolemic rats and fed with 100% standard fed accompanied by giving gemfibrozil 21,6 mg/200-gram rats weight (Positive control)

After 14 days of treatment, the blood cholesterol total, LDL and HDL cholesterol, and triglyceride levels were measured again and recorded as posttest level. Each treatment was made in 5 replications. So the total number of samples is 30 samples.

2.4 Data Analysis

This research model used was a non-factorial randomized group design with the treatment three of 100% rat standard feed, 25% PSP bread mixture, and 50% PSP bread mixture to groups of healthy rats. 100% rat standard feed accompanied by gemfibrozil, 25% PSP bread mixture, and 50% PSP bread mixture against 3 groups of hypercholesterolemic rats. The data was analyzed using Kruskal Wallis on percentage and percentage changes of total cholesterol, triglyceride, LDL, HDL, and body weight. For daily feed consumption, the Shapiro Wilk test was used for the normality test of data.

3 Results and Discussion

3.1 Decrease of total cholesterol levels

Decrease in total cholesterol before and after treatment in rats blood serum can be seen in Table 1.

Table 1. Reduction of total cholesterol

Treatment	Total cholesterol blood serum (mg/dL)		Reduction of total cholesterol levels (%)
	Pretest	Posttest	
A_1	53.40±7.09	50.00±7.31	6.49±1.49
A_2	59.80±4.65	50.60±5.13	15.39±5.51
A_3	54.60±5.03	29.60±1.52	45.54±3.93
A_4	103.20±10.47	72.00±6.89	30.17±2.37
A_5	109.60±12.05	44.20±4.66	59.60±2.44
A_6	92.80±5.16	72.60±4.39	21.72±3.65

The value represents five replicates data ± standard deviation.

The effectiveness of the treatment on reducing total cholesterol in each group of healthy rats can be seen in Table 2.

Table 2. Reduction of total cholesterol levels on healthy rats blood serum

Treatment	Reduction of total cholesterol levels (%)	Mean of rank	P
A_1	6,49±1,49	3,00	0,002
A_2	15,39±5,51	8,00	
A_3	45,54±3,93	13,00	

The value represents five replicates data ± standard deviation.

The effectiveness of the treatment on reducing total cholesterol in each group of hypercholesterolemic rats can be seen in Table 3.

Table 3. Reduction of total cholesterol levels on hyper cholesterole rats blood serum

Treatment	Reduction of total cholesterol levels (%)	Mean of rank	P
A_4	30,17±2,37	8,00	0,002
A_5	59,60±2,44	13,00	
A_6	21,72±3,65	3,00	

The value represents five replicates data ± standard deviation.

P-value is a significant value from the Kruskal Wallis method. If the $P < 0.05$, it indicates a difference in the effect of decreasing total cholesterol between groups due to differences in the treatment received. The rank value shows more specifically about the highest to the lowest impact provided by the treatment. Table 4 and Table 5 show that the most significant percentage reduction in total cholesterol both in the group of healthy rats and hypercholesterolemic rats was found on treatment receiving up to 50% daily bread as a daily feed mixture. In contrast, the lowest was in the control group. PSP bread is still rich in anthocyanin and dietary fiber even though it has lengthy processing. Anthocyanins and dietary fiber are very influential in reducing total cholesterol levels in rat blood serum. According to (4), anthocyanins can inhibit cholesterol synthesis in the liver through the destruction of the HMG CoA reductase enzyme which has a role in synthesizing cholesterol in the liver.

3.2 Decrease of triglycerides levels

Decrease in triglycerides before and after treatment in rats blood serum can be seen in Table 4.

Table 4. Reduction of triglycerides

Treatment	Triglycerides blood serum (mg/dL)		Reduction of Triglycerides levels (%)
	Pretest	Posttest	
A_1	105,60±5,98	96,80±4,49	8,27±2,45
A_2	119,20±12,51	86,20±9,67	27,18±10,25
A_3	152,40±33,57	59,40±13,61	59,38±14,01
A_4	186,00±18,16	96,80±9,03	47,69±5,46
A_5	206,40±36,98	78,20±12,55	61,81±4,76
A_6	172,40±12,63	123,00±9,30	28,34±6,92

The value represents five replicates data ± standard deviation

The effectiveness of the treatment on reducing triglycerides levels in each group of healthy rats can be seen in Table 5.

Table 5. Reduction of triglycerides levels on healthy rats blood serum

Treatment	Reduction of triglycerides levels (%)	Mean of rank	P
A_1	8,27±2,45	3,00	0,002
A_2	27,18±10,25	8,00	
A_3	59,38±14,01	13,00	

The value represents five replicates data ± standard deviation

The effectiveness of the treatment on reducing triglycerides levels in each group of hypercholesterole rats can be seen in Table 6.

Table 6. Reduction of triglycerides levels on hypercholesterole rats blood serum

Treatment	Reduction of triglycerides levels (%)	Mean of rank	P
A_4	47,69±5,46	8,20	0,002
A_5	61,81±4,76	12,80	
A_6	28,34±6,92	3,00	

The value represents five replicates data ± standard deviation

Table 7 and Table 8 show that the highest percentage reduction of triglycerides in rats' blood serum was both on the healthy and hyper cholesterol rats that received treatment with PSP bread as a daily feed mixture up to 50%, while the lowest was in the rats control groups.

According to (5), the content of dietary fiber in purple sweet potato bread was able to bind bile acids so that it can inhibit the formation of triglycerides in the liver. The anthocyanin range in PSP bread can increase the content of lipoprotein lipase in the body, which functions to break the structure of triglycerides produced by the liver.

3.3 Decrease of Low-Density Lipoprotein (LDL)

The reduction of LDL before and after treatment in rats blood serum can be seen in Table 7.

Table 7. Reduction of LDL

Treatment	LDL blood serum (mg/dL)		Reduction of LDL levels (%)
	Pretest	Posttest	
A_1	17,47±6,73	14,44±5,71	17,55±2,10

A_2	25,76±6,20	13,36±4,41	48,74±9,33
A_3	15,52±9,18	0,72±0,36	94,24±3,50
A_4	54,00±10,41	24,44±3,75	53,92±7,89
A_5	55,72±13,22	1,16±1,37	97,86±2,33
A_6	39,12±6,69	19,40±3,85	50,31±6,46

The value represents five replicates data ± standard deviation

The effectiveness of the treatment on reducing LDL levels in each group of healthy rats' can be seen in Table 8.

Table 8. Reduction of LDL levels on healthy rats blood serum

Treatment	Reduction of LDL levels (%)	Mean of rank	P
A_1	17,55±2,10	3,00	0,002
A_2	48,74±9,33	8,00	
A_3	94,24±3,50	13,00	

The value represents five replicates data ± standard deviation

The effectiveness of the treatment on reducing LDL levels in each group of hypercholesterole rats can be seen in Table 9.

Table 9. Reduction of LDL levels on hyper cholesterol rats blood serum

Treatment	Reduction of LDL levels (%)	Mean of rank	P
A_4	53,92±7,89	6,20	0,008
A_5	97,86±2,33	13,00	
A_6	50,31±6,46	4,80	

The value represents five replicates data ± standard deviation

Table 9 and Table 10 show that the highest percentage reduction of triglycerides in rats' blood serum was both on the healthy and hyper cholesterol rats that received treatment with PSP bread as a daily feed mixture up to 50%, while the lowest was in the rats control groups. (6) stated that dietary fiber also has a role in binding LDL directly and helping the disposal of LDL through feces. Apart from the high dietary fiber content, PSP bread also contains antioxidants and anthocyanins, which have a role in preventing the oxidation of HDL by free radical compounds that can turn it into LDL.

3.4 Increase of High-Density Lipoprotein (HDL)

Table 10. Increase of HDL

Treatment	HDL blood serum (mg/dL)		Rise in HDL levels (%)
	Pretest	Posttest	
A_1	14,80±1,30	16,20±2,16	9,18±7,50
A_2	10,20±1,30	20,00±2,73	97,32±28,49
A_3	8,60±1,14	17,00±3,08	98,14±29,30
A_4	12,60±2,30	27,40±4,77	117,90±8,16
A_5	12,00±1,58	28,20±4,08	134,72±9,96
A_6	19,20±1,48	28,60±3,28	49,60±19,24

The value represents five replicates data ± standard deviation.

The effectiveness of the treatment on increasing HDL levels in each group of healthy rats can be seen in Table 11.

Table 11. Increase of HDL levels on healthy rats blood serum

Treatment	Increase of HDL levels (%)	Mean of rank	P
A_1	9,18±7,50	3,00	0,009
A_2	97,32±28,49	10,40	
A_3	98,14±29,30	10,60	

The value represents five replicates data ± standard deviation

The effectiveness of the treatment on increasing HDL levels in each group of hyper cholesterol rats can be seen in Table 12.

Table 12. Increase of HDL levels on hyper cholesterol rats blood serum

Treatment	Increase of HDL levels (%)	Mean of rank	P
A_4	117,90±8,16	8,40	0,003
A_5	134,72±9,96	12,60	
A_6	49,60±19,24	3,00	

The value represents five replicates data ± standard deviation.

Table 13 and Table 14 show that the most significant percent increase in HDL was found in the group of rats that received bread as a daily feed mixture up to 50% in both healthy and hyper cholesterol rats, while the lowest was in the control group. According to (7), the increase of HDL in rat blood serum occurs due to antioxidants that increase APO A1 compounds in the liver. APO A1 is used in the primary formation of HDL compounds in the body. Treatment with bread giving up to 50% showed the highest increase in HDL values in both the healthy rat group and the hypercholesterolemic rat group. In addition, antioxidants in purple sweet potato bread also play a role in preventing the oxidation of HDL to LDL by free radical compounds. Antioxidants will cover every reductive active site in HDL that is damaged due to contamination by free radical compounds.

4 Conclusion

Consumption of purple sweet potato bread as a daily food mix for white male line Wistar rats affected lowering blood cholesterol levels in rats, both in reducing total cholesterol, triglycerides, LDL, and increasing HLD in the blood lipid profile of rats. The most significant effect was given by the feed mixture up to 50%, followed by the provision of bread as a mixed feed as much as 25%, and the lowest was provided by the control treatment.

References

1. Y.P. Hwang, J.H. Choi, H.J. Yun, E.H. Han, H.G. Kim, J.Y. Kim, B.H. Park, T. Khanal, J.M. Choi, Y.C. Chung, H.G. Jeong, **49**, 93 (2011)
2. C.C. Teow, V.D. Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota, G.C. Yencho, **103**, 829 (2007)
3. Hardoko., Herdanto, Lina, S. Marsillam, **20** (2010)
4. E.D. Sigarlaki, A. Tjiptaningrum. **5**, 14 (2016)
5. Fitriani, N. A., Effect of onion extract (Allium ascalonicum) on serum triglyceride levels in hyperlipidemic Wistar rats (in Bahasa Indonesia) (Artikel Hasil Penelitian, 2010).

6. Erizon dan Y. Karani, **5**, 1123(2020)
7. T. Wresdiyati, A.B. Hartanta, M. Astawan, **12**, 126 (2011)