

# International Food Research Journal - Decision on Manuscript ID IFRJ18123

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International Food Research Journal <onbehalf@manuscriptcentral.com>  
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17-May-2018

Dear Mrs. Rosida

Manuscript IFRJ18123, entitled 'Hypocholesterolemic and Hypoglycemic Effects of Modified Water Yam (*Dioscorea alata*) on Wistar Rats', which you submitted to International Food Research Journal, has been reviewed. The comments of the reviewers appear below.

Based on the advice received, your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions. When preparing and submitting your revised manuscript (which should be labelled as such), you will be able to respond to the comments made by the reviewers, the Associate Editor and the Editor in the space provided. You can use this space to document the changes you make to the original manuscript and submit a list of responses to the comments and suggestions, item by item. Your list of responses should also be uploaded as a separate file (i.e. Word) in addition to your comments in the system.

Please note that there is/are comments on the English grammar. Therefore, please make sure to send the manuscript to English Editing Services, and attach the certificate or evidence that you are doing so.

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Thank you for submitting your manuscript to International Food Research Journal; I look forward to receiving your revision. Instructions on how to revise your manuscript are included at the end of this letter.

Sincerely,  
Dr. Faridah Abas  
Editor, International Food Research Journal

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#### Reviewers' Comments to Author:

Reviewer: 1

##### Comments to the Author

This study is interesting but a clear exoalantion along the manuscript should be written well. The title should be rewirte to be more clear an spesific. Cite the most relevant references in the Introduction as well as focus to the topic. Method should be rewrite in more detail, because there is some missed information how to onbtain the data and prepare the modified water yam. Results should be presented in the complete information (for Table and Figures) as well as the result of statistical analysis. Discussion should be presented in the more comprehensive and explore more about the effect of RS3, especially thhis experiment is unusual, hyperchoelsterolemia presumably leads to hyperglycemia. Usually, it is done vise versa. More suggestions cpuld be found in the manuscript

Reviewer: 2

##### Comments to the Author

Material and Methods.

- 1) As the research project reported involved laboratory animals, was the experimental protocol approved by any ethical committee?
- 2) For the plant samples used, are those plants being examined and identified by

trained botanists? if yes, kindly acknowledge the contribution of the botanists.  
3) kindly specify the source of chemicals, reagents and lot number of the testing kits used for biochemical studies.

Results and figures.

- 1) Recommended to use professional statistical diagram software (eg sigma plot, graphpad prism etc) to generate quality figures. The bar and line graph are illegible.
- 2) significance of difference are not indicated anywhere in the graph.
- 3) standard error of mean / standard deviation should be include in all data involving mean.
- 4) the figure legends captions are too brief.

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## Hypocholesterolemic and hypoglycemic effects of modified water yam (*Dioscorea alata*) on hypercholesterolemic Wistar rats

### Abstract

This study was inspired due to the potential use of water yam as functional food. This is because it has both high starch and resistant starch. In Indonesia, water yam is differentiated by its flesh color, such as purple, yellow and white. The modified water yam flour was made by three-cycle autoclaving-cooling process in order to increase the resistance of the starch content (RS type 3). The objective of this study was to evaluate the hypocholesterolemic and hypoglycemic effect of modified water yam in hypercholesterolemic rats. The study was carried out using 32 Wistar rats. They were made hypercholesterolemic by a certain diet that contained high lipid and cholesterol and were divided into four groups. Each group was fed with different diet such as which were purple, yellow and white autoclaved water yam flour and standard diet (AIN93) as a control, respectively. The treatments were assigned for four weeks and every week the serum lipid profile and glucose levelcontent were analyzed. The results showed that rats which were fed with modified water yam flour had lower concentration of total cholesterol, LDL cholesterol, triglyceride, and blood glucose than those which were fed with standard diet (AIN93). The higher reduction of total cholesterol and glucose level (approximately—39.8% and 58.1% respectively) was found in the group fed with modified white water yam. It can be concluded that modified white water yam was proved to pose

**Comment [TE1]:** Should be specified, what type of modification was used. Autoclaved?

**Comment [TE2]:** Make a scientific statement. For example: yams contain high resistant starch therefore they are suitable for functional foods

**Comment [TE3]:** What is the correlation between cholesterol level and glucose level in hypercholesterolemia? Usually it is used conversely, diabetic/hyperglycemia condition leads to hypercholesterolemia. Please explain your reason and the scientific justification

**Comment [TE4]:** Simultaneously?

**Comment [TE5]:** Explain why? Also explain how did the effects of purple and yellow water yam?

hypocholesterolemic and hypoglycemic effects and could be developed as functional food. The findings of this research can facilitate the development of functional food for the healthiness of human, and for diabetic and hypercholesterolemic patients.

**Keywords:** modified water yam, hypocholesterolemic, hypoglycemic.

**Comment [TE6]:** Add two more key words

## Introduction

~~Indonesia had various traditional foods which were potential to be developed as functional food; however they were not used optimally. One of the postharvest which had not yet used optimally was water yam. Water yam (*Dioscorea alata*) is known as Ubi Kelapa or Uwi in Indonesia. Water yam is usually differentiated by its flesh color, such as white, yellow and purple water yam.~~

**Comment [TE7]:** Think globally not only think from the perspective of a country

Water yam is usually boiled or steamed and consumed as staple food in village society. Being processed, Resistant Starch type III (retrograded starch) is formed and it has positive effect to human health. The previous study revealed that three cycle treatment of arrowroot starch increased resistant content of 10.91% (Sugiyono *et al.*, 2009). Autoclaving-cooling treatments of water yam resulted in a significant increase in crystallinity of the starches, swelling power, and water absorption capacity (Rosida *et al.*, 2017) Autoclaving-cooling cycle could produce more retrograded amylose fraction (re-crystalization) and was known as RS3

**Comment [TE8]:** This sentence is not clear

formation (Mutungi *et al.*, 2009). Retrograded amylose (RS3) had heat-stable properties, very complex and resistant to amylase enzyme.

So *et al.* (2007) reported that feeding RS3 to experimental rats had significant effect on lipid metabolism. Anderson *et al.* (2009) stated that an effort in decreasing blood cholesterol level was to consume high dietary fiber food. Dietary fiber can inhibit cholesterol absorption in the intestine so that reducing cholesterol level in the blood and increasing cholesterol excretion in the feces.

Hypercholesterolemia is a condition which cholesterol level in blood plasma over its normal limit, that is over than 200mg/L whereas LDL level is over than 130mg/dL and HDL level is lower than 40mg/dL. The high cholesterol level in the blood serum cholesterol was one factor which had the risk of cardiovascular disease (Cheng and Lai, 2000). Cholesterol level in the blood was influenced by many factors, such as the amount and kind of fat intake, dietary fiber intake, exercise, and so on. The cholesterol reduction could be done by therapeutic diet. Some of dietary fiber and resistant starch could lower LDL cholesterol level. So that consuming high resistant starch food in sufficient quantity could give healthy effect to human body (Anderson *et al.*, 2009)

Hernawati *et al.* (2013) reported that dietary fiber from rice bran could reduce serum cholesterol total, triglycerides, and LDL level and increased HDL level of hypercholesterolemic rats. Additionally, it supported blood glucose level balance especially to the type 2-diabetic mellitus patients. The consumption of dietary fiber diet intensively in long period of

**Comment [TE9]:** It is not a relevant reference

**Comment [TE10]:** What of the correlation between hypercholesterolemia and glucose level? Not vise versa. Does hypercholesterolemia lead to hyperglycemia? What is your scientific reason?

times could reduce serum glucose level, by slowing down of glucose absorption and insulin response. The soluble fiber retarded glucose diffusion and delayed carbohydrate absorption and digestion. So it had positive effect to lower glucose absorption rate so the body had never suffered hyperglycemic condition.

The aim of this study was to evaluate the hypocholesterolemic and hypoglycemic effects of modified water yam in hypercholesterolemic rats. Presumably, the effect in reducing blood glucose level was due to some polysaccharides in water yam, such as resistant starch and dietary fiber.

## **Materials and methods**

### *Materials*

Selected purple, yellow, and white water yam tuber (*Dioscorea alata*) were heated in an autoclave for 15 minutes (120°C) and cooled in a refrigerator for 24 hours (4°C). The autoclaving-cooling treatment was repeated up to 3 times prior to slicing, drying and milling in order to make modified water yam flour. Reagent used in this research were analytical grade, such as cholesterol kit, glucose kit, HDL precipitate, ethanol.

### *Methods*

Selected 32 male Wistar rats (*Ratus norvegicus* Wistar) 2-3 months of age weighing 150-225g were used in the experiment. Acclimatization was conducted for three days with standard feed of AIN93 (Reeves *et al.*, 1993). The AIN93 standard diet was consisted of corn starch (670,7g/kg),

**Comment [TE11]:** Give more explanation how the prepare autoclaved modified water yam

casein (140g/kg), sucrose 9100g/kg), soybean oil (40mg/kg), AIN mineral mix (35mg/kg), AIN vitamin mix (10mg/kg), choline bitartrate (2.5g/kg), L-cystine (1.8g/kg), except carboxymethylcellulose addition (fiber-free diets).

After that the rats were fed hypercholesterol diets for seven days, i.e standard feed which was supplemented with 1% cholesterol and PTU (prophyl thio uracyl), so that all the rats were suffered hypercholesterolemic (cholesterol total over than 200mg/dL). The rats were randomly assigned into four groups of eight rats per group. Rats in group 1 served as control and fed standard diet (AIN93), while group 2, 3, and 4 were fed purple, yellow and white modified water yam flour respectively. The treatment diets and water were given *ad libitum* for four weeks. The measurement was performed on days 0, 7, 14, 21 and 28. Blood were taken from a retro-orbital plexus after fasting for 16 hours and were measured for cholesterol total and HDL by CHOD-PAP method; blood Triglyceride by GPO-PAP method and blood glucose by the GOD/PAP method. LDL was measured by Friedewald formulation as followed:

$$\text{LDL} = \text{cholesterol total} - (\text{HDL} + \text{TG})$$

5

All data were analyzed by Analysis of Variance (ANOVA) and followed by Duncan Multiple Range Test (DMRT's).

## Results and discussion

**Comment [TE12]:** How to make hypercholesterol diet? Is this the right terminology? What is the difference with atherogenic diet?

**Comment [TE13]:** Ad libitum? Or formulated into the dieat? It is ot clear. The rats only fed by water yam flour?



### *Resistant starch and dietary fiber content*

Modification process by autoclaving-cooling cycles in order to increase RS content was reported by many researchers, such as Zabar *et al.* (2008) and Sugiono *et al.* (2009). In this research, purple, yellow and white water yam flour had resistant starch content of 7.55%, 7.14%, 9.04% respectively. The previous study revealed that water yam modification by three cycle autoclaving-cooling treatment was able to increase RS and DF content, thus able to decrease blood glucose level (Rosida *et al.*, 2016).

**Comment [TE14]:** Explain in the method section how to measure RS

**Comment [TE15]:** In hyperglycemia condition?

### *Cholesterol total*

After 28 day intervention, serum cholesterol total of purple, yellow, and white water yam group decreased by 39.44%, 37.33% and 39.87%, respectively. However, those of control group were constant (201.5 - 203.8mg/dL). The data proved that modified water yam flour had potency to reduce blood cholesterol due to its resistant starch and dietary fiber content. So *et al.* (2007) reported that feeding RS3 to experimental rats had significant effect on lipid metabolism. Resistant starch could arrive in the colon without changing and had the function as dietary fiber.

**Comment [TE16]:** Explore more how the role of RS in modulating lipid profile

One method in reducing blood cholesterol level was to consume more dietary fiber diet. Dietary fiber diet could inhibit cholesterol absorption in the small intestine and finally reduce cholesterol level in the blood plasma and increase cholesterol production in the liver, bile acid production and cholesterol excretion in the feces. So that dietary fiber had been used

**Comment [TE17]:** What is the different effect between RS and dietary fiber? Focus on RS, not make a wide discussion by explain dietary fiber/

intensively and recommended to keep blood cholesterol level at normal level (Anderson *et al.*, 1994).

#### *Triglyceride (TAG), HDL and LDL level*

At the beginning, after hypercholesterolemic diet intervention, triglyceride, LDL and HDL level of all rats were not different significantly ( $p < 0.05$ ). However, after feeding treatment, those of modified water yam flour groups were lower than standard diet group. The reduction of cholesterol (Fig1) was in accordance with triglyceride and LDL level reduction (Fig2). It indicated that modified water yam flour had potency in lowering blood cholesterol, LDL and triglyceride level. This phenomenon revealed that modified water yam flour had hypocholesterolemic effect due to its resistant starch and dietary fiber content.

The high fiber and high resistant starch diet could increase LDL receptor activity in the liver. This activity fulfilled the availability of tissue cholesterol, so more blood cholesterol was used that reduced blood cholesterol level (Anderson *et al.*, 2009)

#### *Blood glucose level*

Statistically, there was a significant decrease ( $p < 0.05$ ) in blood glucose level during 4 week feeding experiment. After 28 day intervention, the serum glucose of purple, yellow and white water yam group reduced by 49.88%, 53.62%, and 58.10%, respectively, while those of standard group were constant (146.01-148.98mg/dL). The data showed that modified water

**Comment [TE18]:** Explore the proposed mechanism of lipid profile improvement based on scientific/previous studies by others

yam flour was potential in decreasing serum glucose level due to its resistant starch content. The availability of resistant starch in small intestine could lower glycemic and insulemic response on diabetic patients (Okoniewska and Witwer, 2007).

Some factors which probably influenced the blood glucose reduction were dietary fiber and resistant starch. Dietary fiber, especially soluble fiber, had viscous properties and could decrease glucose response (Marsono, 2002). The plan could serve as a great therapeutic diet in the management of diabetes.

### **Conclusion**

The research revealed that rats which were fed with modified water yam flour had lowered cholesterol total, LDL and glucose and higher HDL level than those of control rats with AIN93 standard diet. The modified white water yam flour had higher reduction in the cholesterol and glucose level due to its high RS and Dietary Fiber content. It can be concluded that modified water yam flour has hypocholesterolemic and hypoglycemic effect on experimental rats.

### **Acknowledgement**

This study was funded by The Ministry of Research, Technology and Higher Education, General Directortate of Research Support and Development, Republic Indonesia.

**Comment [TE19]:** Explain the mechanism of blood glucose level reduction and what is the effect of different type of water yam? Why white water yam had the most pronoun effect?

## References

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Marsono. 2002. Hypoglycemic and hypolipidemic properties of edible podded peas (*Pisum sativum* Linn) and soybean (*Glycine max* Merr) diets in aloxan induced diabetic spargue dawley rats. *Agritech* 6 22(4): 137-143.

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Comment [TE20]: Use high traceability letartures

Mutungi, C., Rost, F., Onyango, C., Jaros, D. and Rohm, H. 2009. Cystalinity, thermal and morphological characteristics of resistant starch type III Produced by hydrothermal treatment of debranched cassava starch. *Starch/Starke* 61(11): 634-645.

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Sugiyono, Pratiwi, R., and Faridah, D. N. 2009. Arrowroot (*Marantha arundinaceae* L) starch modification through autoclaving-cooling cycling treatment to produce resistant starch type III. *Jurnal Teknologi dan Industri Pangan* 20(1): 17-24.

Zabar, S., Shimoni, E., and Peled, H. B. 2008. Development of nanostructure in resistant starch type III during thermal treatment and cycling. *Journal of Macromolecule Bioscience* 8(2): 163-170.

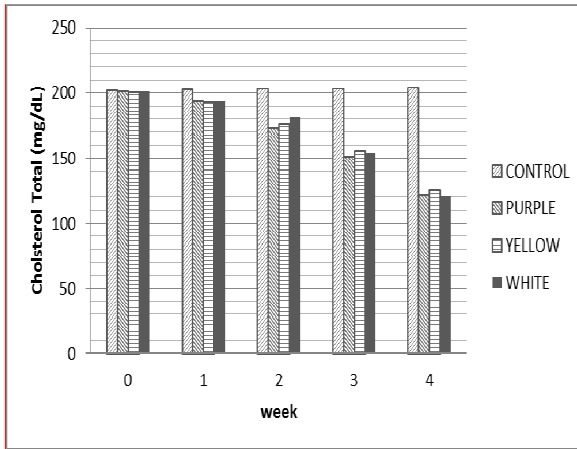
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**Table 1.** Resistant Starch and Dietary Fiber content of modified water yam

flour

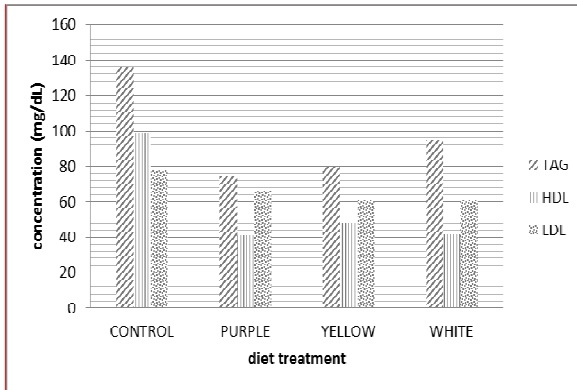
<b>Water Yam Flour</b>	<b>Dietary Fiber (%)</b>	<b>Resistant Starch (%)</b>
Purple	13.04	7.55
Yellow	13.42	7.14
White	13.81	9.04

**Comment [TE21]:** Put standard deviation and statistically significance



**Comment [TE22]:** Put error bar and statistically notation

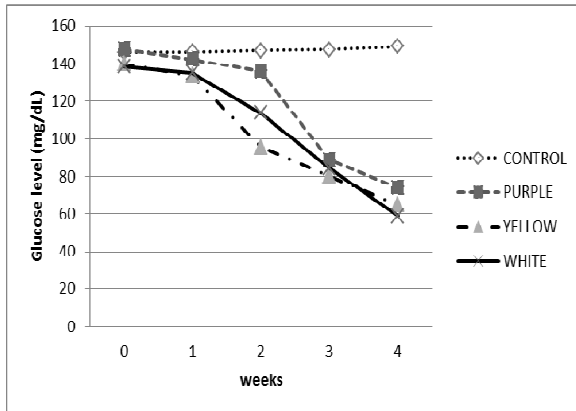
**Fig 1.** Changes of serum cholesterol total of rats after 28 day intervention by standard diet and modified water yam flour diets



**Comment [TE23]:** Similar to Fig 1.

**Fig 2.** Triglyceride (TAG), HDL and LDL level of hypercholesterolemic rats after 28 days intervention by standard diet and modified water yam flour diets.





**Fig 3.** Changes of blood glucose level of hypercholesterolemic rats during 4 week feeding of standard diet and modified water yam flour diets

**Comment [TE24]:** Similar to Figure 1

18-Jul-2018

Dear Mrs. Rosida:

Manuscript ID IFRJ18123.R1 entitled "Hypocholesterolemic and Hypoglycemic Effects of Modified Water Yam (*Dioscorea alata*) on Wistar Rats" which you submitted to International Food Research Journal, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/upm-ifrj> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

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10-Aug-2018

Dear Mrs. Rosida

Thank you for sending the revised version of this paper ('Hypocholesterolemic and Hypoglycemic Effects of Modified Water Yam (*Dioscorea alata*) on Wistar Rats'). I have now had the opportunity to examine your revised manuscript and I am pleased to accept it for publication in International Food Research Journal. Kindly refer to the attachment for the acceptance letter.

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Thank you again for your contribution to the Journal.

Sincerely,  
Dr. Faridah Abas  
Editor, International Food Research Journal

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Dear Author,  
ACCEPTANCE LETTER

The Editorial Team of International Food Research Journal, is pleased to inform you that your manuscript has been accepted for publication. We thank you for your contribution to the International Food Research Journal and encourage you to submit other articles to the Journal.

Please note that all manuscripts must be accompanied by a signed copyright agreement form to enable the manuscript to be processed for publication. You are requested to send the copyright agreement form within three (3) working days from the date of the email. Please print, fill and sign the attached copyright agreement form and send the scanned copy to [ifrj@upm.edu.my](mailto:ifrj@upm.edu.my)

Thank you.

Yours sincerely,  
Professor Dr. Son Radu  
Editor

International Food Research Journal