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Effect of Administration of CHAGURO Made of Chayote (*Sechium edule*) and Tuna (*Thunnus* sp.)on Rats Induced with Streptozotocin-Nicotinamide and a High-Fat Diet

TOTO SUDARGO[1]*, BIANDA AULIA[1], ATIKA ANIF PRAMESWARI[1], ALIM ISNANSETYO[2], INDUN DEWI PUSPITA[2], SITI ARI BUDIYANTI[2], RAHADYANA MUSLICHAH[1], TIRA ARISTASARI[1], SHEILA ROSMALA PUTRI[2] and KHUSNUL ALFIONITA[2]

[1]Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[2]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Abstract

This study was conducted to develop Chaguro, a low-cost supplementary food made of chayote (*Sechium edule (Jacq.) Swartz*) and tuna fish (*Thunnus* sp.), for diabetes and dyslipidemia diet therapy. In order to find a formula with effective hypoglycaemic and antidyslipidemic properties, dried tuna and chayote were mixed at different ratios: F1 (75% tuna, 25% chayote), F2 (50% tuna, 50% chayote), and F3 (25% tuna, 75% chayote). Thirty male Sprague Dawley rats were assigned into healthy control group or groups induced with streptozotocin-nicotinamide and a high-fat diet. Chaguro was administered 2.7 g/ kgBW/ day using a gavage for 28 days. The administration of all Chaguro formulas improved blood markers compared to the negative control group($p < 0.001$). Chaguro F1 lowered fasting blood glucose (97.07±1.18 vs 266.31±5.31), total cholesterol(113.59±2.22 vs 208.78±4.31), triglycerides (89.93±2.51 vs142.35±2.83), LDL-c (33.87±1.87 vs 87.85±3.34) and increased HDL-c (69,08±1,85 vs 23,91±1,64) level the most compared to the negative control group ($p < 0.001$). Streptozotocin-induced weight loss was also prevented in all diabetic rats fed with Chaguro, with the body weight being similar to that of healthy controls at the end of the intervention ($p < 0.001$). This study found that Chaguro may be a potential food product to help lower blood glucose and improve lipid profile in diabetes and dyslipidemia.

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CONTACT Toto Sudargo toto_sudargo@yahoo.co.id Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

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Introduction

Diabetes is an increasing metabolic problem that contributes to almost 4.2 million of global deaths in 2019.[1] The worldwide prevalence of diabetes was predicted to be 10.2% in 2030.[2] In 2018, 2.0% of adults were diagnosed with diabetes in Indonesia.[3] In addition, around 19.7% of the population were estimated to have impaired glucose tolerance according to 2018 Indonesia's Basic Health Research (Riskesdas).[3]

Type 2 diabetes mellitus (DM) has been widely studied as a major risk factor for cardiovascular diseases (CVD), which is currently the number one cause of mortality worldwide.[4] In 2018, the prevalence of CVD in Indonesia was 1.5%.[3] CVD accounts for 52% of deaths in Type 2 DM and 44% in Type 1 DM.[5] Adults with type 2 diabetes mellitus are more than twice as likely to develop CVD than adults without diabetes mellitus.[6] It is estimated that more than 70% of individuals with diabetes mellitus will die from CVD.[6] Among many predictors, lipid markers are the most commonly used indicator of CVD risk.[7]

In terms of diet therapy for chronic disease and diabetes management, there is a growing trend in the use of natural compound or food with bioactive properties to help improve metabolic conditions.[8] Functional food refers to food with basic nutritional impact, which also has beneficial effects on one or more functions of the human organism; it either improves the general and physical conditions and/or decreases the risk of the evolution of diseases.[9]

Chayote or *Sechium edule*, a plant from the Cucurbitaceae family, is known for its economic value for trade and for its culinary applications in many countries due to its nutritional content.[10] Chayote is also used for medicine and complementary treatment, such as for hypertension or dissolving kidney stones.[11] The administration of chayote extract was reported to lower blood glucose and improved lipid profile in diabetic rats.[12,13] Chayote juice has also been reported to significantly reduce starch-induced postprandial glycemic load even in the absence of carbohydrate digestive enzymes' inhibitory activities.[14] Feeding rats with chayote juice for 3 months along with sucrose or fructose-sweetened beverages not only decreased the progression of impaired glucose tolerance but also significantly prevented development of oxidative stress by maintaining total antioxidant potentials in the blood.[15]

Fish consumption reduces chronic non-communicable diseases such as cardiovascular diseases, rheumatoid arthritis, and several cancer types.[16] Diversification of protein sources may modulate insulin resistance in different manners. Based on studies in rats and human subjects, the intake of fish proteins was believed to have favorable effects on glucose tolerance and insulin sensitivity.[17,18] Tuna fish (*Thunnus s* sp.) is a high-protein food known for its bioactive compounds such as omega-3 fatty acids which exhibit beneficial effects, including its hypocholesterolemic, antiatherosclerosis, and anticancer properties.[19,20]

Previous investigations have shown the health benefits of chayote and tuna independently, but the findings were mostly limited to the potential of isolated bioactive compounds or extract.[12–15,20–22] In this study, we aimed to combine tuna and chayote to develop *Chaguro*, a food product that may improve blood glucose and lipid profile in diabetes and dyslipidemia. This animal study was conducted to determine the most effective *Chaguro* formula to be developed in further study.

Methods

Preparation of *Chaguro*

Fresh raw tuna fish (*Thunnus* sp.) was obtained from a local market in Yogyakarta. The flesh was separated from the bone and cooked for 25 minutes using a steamer, after which it was shredded before oven-dried for 6 hours using a temperature of 65°*C*.The dried meat was then blended intosmaller particles ofaround 0.1 mm in diameter.

Sechium edule (chayote) obtained from a local market in Yogyakartawas washed, peeled, and cut into slices of around 0.3 cm thick. The drying process was performed using an oven with a temperature of 55°*C* for 4 hours. Afterward, the dried slices were blended into small flakes.

The dried products of tuna and chayote were mixed together with different ratios for the intervention: 1) *Chaguro* F1 (75% tuna, 25% chayote), *Chaguro* F2 (50% tuna, 50% chayote), *Chaguro* F3 (25% tuna, 75% chayote).

Analysis of Macronutrient and Mineral Content
Analysis of macronutrients and mineral content were carried out in Chemix Pratama Laboratory, Yogyakarta, Indonesia. Dry ashing method was used to determine total ash content; samples were combusted using a muffle furnace at a temperature of 550°*C*.[23] Total protein content of samples was estimated using Micro-Kjeldahl method.[24] Total fat content was determined by the Soxhlet extraction method.[23] Carbohydrate content was estimated by difference (subtracting the measured protein, fat, and ash from the total weight). Crude fiber was determined by boiling the sample with 1.25% dilute H_2SO_4, washing it with water, and boiling it further with 1.25% dilute NaOH. The remaining residue after digestion was the crude fiber. This method followed the guidelines described byAssociation of Official Analytical Chemists (AOAC).[23] Calorie value (kcalper 100 g) was estimated by converting macronutrient content (protein, fat, carbohydrate) into energy using conversion factors of 4 kcal/g for protein and carbohydrate, and 9 kcal/g for total fat.[25] Mineral content (Ca, Mg, Mn, Zn) was estimated using atomic absorption spectroscopy (Perkin-Elmer Model 3110., Germany) according to the manufacturer's manual. All measurements were repeated twice.

Experimental Animals
Thirty male Sprague Dawley rats (160—180 g) aged 8 weeks were obtained from the Experimental Animal Laboratory of Food and Nutrition Postgraduate Research Center, Universitas Gadjah Mada. All rats underwent an acclimatization period of seven days before the intervention. They were given the AIN-93 Mstandard diet (formulated by American Institute of Nutrition in 1993 for adult rodents) and water *ad libitum*. Throughout the experiment, all rats were kept inside a stainless-steel cage in a humidity-controlled room with a temperature of 20-24°C and received a 12/12-h dark-light cycle per day.

After the adaptation period, the rats were randomly divided into five groups with six rats each: 1) Healthy control: healthy rats that received no supplementation, 2) Negative control: rats induced with diabetes and dyslipidemia that received no supplementation, 3) F1: Diabetic and dyslipidemic rats that received *Chaguro* F1 (25%chayote, 75% tuna),4) F2: Diabetic and dyslipidemic rats that received *Chaguro* F2 (50%chayote, 50% tuna), and 5) F3: Diabetic and dyslipidemic rats that received *Chaguro* F3 (75%chayote, 25% tuna).

The induction of dyslipidemia to negative control, F1, F2, and F3 groups was performed by daily oral administration of 3 ml high-fat food (a mix of beef fat, egg yolk, and water) for 2 consecutive weeks. After that, diabetes was induced with an Intraperitoneal injection of 45 mg/kg streptozotocin (STZ) prepared in 0.1 mol/L citrate buffer (pH 4.5), followed by an injection of 110 mg/kg nicotinamide (NA) diluted in saline solution.[26]

Intervention period with *Chaguro* was conducted for 28 days. The dosage for *Chaguro* administration was 2.7 g/ kgBW/ day. As this was a preliminary study, the dose was based on the assumption that the planned supplementation of *Chaguro* for human is 30 g per day. The dose for rat was calculated with a conversion factor of 0.018 from a human weighing 70 kg.[27] All *Chaguro* formulas were blended and diluted in water for administration using an oral gavage to rats.

Blood collection was performed after diabetes and dyslipidemia induction and after the intervention ended. Blood was collected from retro-orbital plexus and allowed to coagulate for 30 minutes. Serum was obtained by centrifugation of the coagulated blood at 1400×g for 15 min and then stored at−20°C for measurements. Ethical approval for this animal study was obtained from the Ethical Committee of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (KE/1234/10/2019).

Serum Glucose and Lipid Profile Measurement
The serum levels of glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic colorimetric methods (Diasys Diagnostics, Germany). All procedures were performed according to the manufacturer's instructions.All measurements were repeated twice.

Statistical Analysis
Data are presented as mean ± standard deviation. A normality test was performed before performing comparative analysis. One-way ANOVA followed by post-hocTukey HSD test was used to Compare blood glucose, lipid profile, and body weight between

groups at different measurement times. A two-sided p-value of < 0.05 was considered statistically significant.

Results

Nutrient Content

Table 1 shows the nutrient content of all *Chaguro* formulas. The highest calorie content was found in *Chaguro* F1 (373.32 kcal), followed by F2 (341.03 kcal),and F3 (244.91 kcal). F1 formula contained the highest percentage of dried tuna and had the highest protein content (66.98%). The highest crude fiber and carbohydrate content were found in *Chaguro* F3, the formula with 75% dried chayote. The calcium content of the formulas ranged from 146.14 to 157.56 mg per 100 g. *Chaguro* F3 had the highest magnesium (149.44mg/100g), while F1 had the highest manganese content (4.70 mg/100g). The zinc content of the formulas ranged from 1.59 to 1.73 mg/100g product.

Table 1: Macronutrient and mineral content of Chaguro formulas per 100 gram

	Chaguro F1 (75% tuna, 25% chayote)	Chaguro F2 (50% tuna, 50% chayote)	Chaguro F3 (25% tuna, 75% chayote)
Calories (kcal)	373.32±0.31	341.03±1.58	244.91±0.99
Protein (g)	66.98±0.06	52.52±0.08	28.75±0.07
Fat (g)	8.07±0.01	9.69±0.18	6.31±0.11
Carbohydrate (g)	19.42±0.10	30.39±0.30	59.80±0.24
Crude fiber (g)	7.73±0.02	9.81±0.26	10.740±0.10
Ash (%)	5.53±0.16	7.40±0.05	5.14±0.06
Calcium (mg)	146.14±3.40	157.56±2.46	146.97±1.18
Magnesium (mg)	116.74±0.55	107.34±0.59	149.44±0.57
Manganese (mg)	4.70±0.07	1.10±0.07	1.12±0.10
Zinc (mg)	1.59±0.01	1.60±0.03	1.73±0.03

Values are the means from two measurements± standard deviation.

Table 2: Fasting blood glucose (FBG) of rats before and after supplementation with Chaguro

Group	FBG level (mg/dL)	
	Pre-test	Post-test
Healthy control (n=6)	69.92±2.11^{a}	71.19±1.96^{a}
Negative control (n=6)	262.62±5.77^{b}	266.31±5.31^{b}
F1 (n=6)	260.35±6.58^{b}	97.07±1.18^{c}
F2 (n=6)	262.01±6.04^{b}	109.34±1.53^{d}
F3 (n=6)	261.88±3.33^{b}	129.21±3.25^{e}
p	<0.001	<0.001

FBG, fasting blood glucose; F1: 75% tuna, 25% chayote; F2: 50% tuna, 50% chayote; F3: 25% tuna, 75% chayote
Data are presented as mean ± SD; In each column, data with different superscripts are statistically significant at p <0.05 (one-way ANOVA and Tukey HSD)

Blood Glucose

Before the treatment, all rats induced with diabetes had a higher fasting blood glucose level compared to that in the healthy control group (261.7 ± 5.3 vs 69.9 ± 2.2) (Table 2). This level of blood glucose was within 200 mg/dL-450 mg/dL range, indicating a Stage 1 Diabetes in rats.[28] At the end of the treatment, all treated group had significantly lower blood glucose compared to the negative control group ($p < 0.001$). Diabetic rats treated with *Chaguro* F1 showed the greatest reduction in blood sugar level, even though it is still higher compared to that in normal control ($p < 0.001$).

Lipid Profile

The effect of treatment on serum lipids of rats is presented in Table 3. There was a significant difference between groups after *Chaguro* administration ($p <0.001$). A greater reduction in total cholesterol was observed in the F1 group. In accordance, the greatest improvement for other lipid profile components (TG, LDL-c, HDL-c)was also seen in the F1 group compared to other formulas (*Chaguro* 2 and 3). The posthoc Tukey HSD test showed that none of the lipid profile levels in treatment groups were statistically similar to that of healthy control at the end of the intervention.

Bodyweight

Figure 1 shows the change in body weight through out the intervention. The initial body weights slightly differed among groups ($p < 0.01$) with the healthy control being the lowest: 188.33±3.01, 194.83±3.60, 193.33±2.80, 192.16±2.78, and 195.16±3.86g for healthy control, negative control, F1, F2, and F3, respectively. At the end of week 2, the mean body weight of negative control group was significantly different from that of the other groups according to one-way ANOVA and post-hoc Tukey analysis ($p< 0.001$). This trend continued until the end of the experiment.

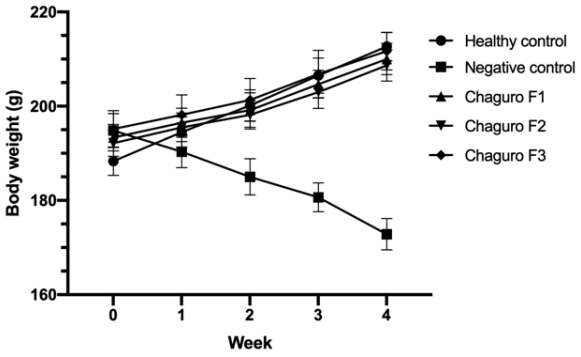

Fig.1: Body weight change throughout the intervention. The body weight (mean ±SD) of each group of rats (n = 6 per group) was recorded weekly

Discussion

Several studies have highlighted the health benefits of tuna and chayote including its potential in improving metabolic disturbance.[12,22,29,30] However, this study is the first to develop a food product from these resources and evaluate the combined effect on blood glucose and lipid profile. Our study found that all *Chaguro* formulas improved the condition of diabetic and hyperlipidemic rats, even though it went through drying processes which might reduce its nutritional value.[11,31]

Regarding effectiveness, *Chaguro* F1 exhibited the most significant improvement of all blood parameters. Omega-3 fatty acids found in tuna, which has been widely studied as a cardiovascular protective nutrient,[32] might be responsible for lowering TC, TG, LDL-c, as well as increasing HDL-c level in diabetic rats. One of the direct modulatory effects of omega-3 in repairing lipid metabolism is through the activation of peroxisome proliferator-activated receptor (PPAR) which subsequently reduces the secretion of TG, increased fatty acid oxidation, and improved insulin sensitivity,[33] In this study, the formula with the highest ratio of tuna increased the HDL-c level by 181%. It was likely that the omega-3 polyunsaturated fatty acids played a direct role in

modifying HDL composition and function which results in increased HDL maturation.[34,35]

Various cooking processes affected omega-3 in tuna differently. A study found that cooking tuna by boiling at 100°C had a minimal effect (1% reduction) on its omega-3 composition, but frying destroyed the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) abundantly.[31] In this study, despite the use of lower temperature to dry the tuna, the long cooking time might lead to a higher degradation of fatty acid content.

The higher protein content in F1 was also likely to play a major role in altering blood results. This is in line with a previous investigation in subjects with type 2 diabetes that reported adopting a high-protein diet for 6 weeks, especially from animal sources, promoted improvement of glucose and fat metabolism.[36] Furthermore, fish protein has been shown to have health-promoting effects due to its antioxidant, angiotensin-converting enzyme inhibitor, and antidiabetic properties.[21,22,37] A study showed that rats fed with salmon protein hydrolysate in substitute of 25% casein protein in their diet had better postprandial glucose regulation than those fed with 100% protein from casein, despite the similar protein content in the feed.[21] The possible mechanism of marine peptides as an antidiabetic agent is through the upregulation of insulin signaling pathway which in turn improved the activity of glucose uptake, hence reducing blood glucose levels.[30]

Even though *Chaguro* F1 was more effective than F2 and F3, when compared to the negative control, administration of F3 (25% tuna, 75% chayote) still resulted in a marked reduction of blood glucose level by 51%.This is supported by a previous study that showed decreased blood glucose and improved lipid profile in diabetic rats after oral administration of 100 and 200 mg/kg BW ethanolic extract of chayote fruit.12 In our study, however, we dried the fruit and subject it to 55℃ exposure of heat. Loizzo *et al*. reported that cooked chayote pulp had approximately half the total phenols content of the raw version, while these phytochemical components were known to positively correlate with inhibitory activities of carbohydrate-hydrolyzing enzymes.[11] None the less when compared to other cooking processes, roasted pulp showed the highest antioxidant activity according to the 2,2-diphenyl-1 picrylhydrazyl (DPPH) radical scavenging test.[11] It can be implied that the improvement of metabolic abnormalities in this study might also be attributed to the phytochemical content of *S. edule*.

Table 3: Lipid profile among groups before and after supplementation with Chaguro

Group	Total Cholesterol (mg/dL)		Triglyceride (mg/dL)		HDL-C (mg/dL)		LDL-C (mg/dL)	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Healthy control (n=6)	88.88±3.06^{a}	92.05±4.00^{a}	78.22±2.08^{a}	80.13±2.73^{a}	86.64±1.28^{a}	84.60±1.76^{a}	23.24±1.73^{a}	24.96±1.87^{a}
Negative control (n=6)	206.41±4.79^{b}	208.78±4.31^{b}	140.02±2.41^{b}	142.35±2.83^{b}	25.00±1.28^{b}	23.91±1.64^{b}	85.97±3.37^{b}	87.85±3.34^{b}
F1 (n=6)	210.19±6.33^{b}	113.59±2.22^{c}	141.60±3.63^{b}	89.93±2.51^{c}	24.54±0.80^{b}	69.08±1.85^{c}	84.62±2.61^{b}	33.87±1.87^{c}
F2 (n=6)	214.08±5.64^{b}	123.70±1.01^{d}	144.03±1.57^{b}	98.56±3.27^{d}	24.42±1.71^{b}	62.46±1.89^{d}	87.54±2.33^{b}	38.05±2.89^{c}
F3 (n=6)	212.60±6.52^{b}	137.78±2.51^{e}	143.18±3.24^{b}	111.89±2.19^{e}	24.20±1.82^{b}	52.54±2.01^{e}	88.21±1.59^{b}	49.79±1.96^{d}
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

HDL-C: High-density lipoprotein cholesterol ; LDL-C: Low-density lipoprotein cholesterol
F1: 75% tuna, 25% chayote; F2: 50% tuna, 50% chayote; F3: 25% tuna, 75% chayote
Data are presented as mean ± SD; In each column, data with different superscripts are statistically significant at p <0.05 (one-way ANOVA and Tukey HSD)

STZ induction causes pancreatic B-cell damage which leads to inhibition of proinsulin synthesis.[26] The severe loss of body weight in diabetic rat model might results from protein wasting because of the significant reduction of glucose uptake.[38] Oral administration of three different *Chaguro* formulas for 28 days maintained the bodyweight of diabetic rats to a level similar to healthy controls. This might be due to the additional calorie and protein intake from *Chaguro* supplementation which contributed to the reversal effect of STZ-induced weight loss. It has also been discovered in a prior study that *S. edule* fruit extract could prevent bodyweight loss after diabetes induction.[12]

This study is among those that attempt to administer the near-final form of a food product, not the extracted bioactive compounds, to animal models and showed positive outcomes. Our findings provide evidence for future research in functional food development, particularly about the potential of tuna and chayote as low-cost food resources and the use of simple drying methods to prolong shelf-life. This preclinical study also serves as a baseline for subsequent research in human subjects. Nevertheless, further development in food processing may be required to better preserve the nutritional quality and bioactive compounds of the product and to ensure sensory acceptability among target populations.

Conclusion

Our results indicate that the administration of *Chaguro* F1 (75% Tuna, 25% Chayote) produced the closest FBG, TC, TG, HDL-c, and LDL-c to that of normal control at the end of the intervention. We therefore suggest the combination of 75% dried tuna and 25% dried chayote as an effective supplemental food to reduce the risks of metabolic disease and help manage diabetes and dyslipidemia.

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Author Contributions: T.S., A.I, I.D.P, S.A.B, and R.M conceived and designed the study; T.S., T.A., S.R.P, and K.A. performed the experiments and collected the data; T.S., B.A., and A.A..P analyzed the data; T.S., A.I, I.D.P, and S.A.B provided materials; T.S., B.A, and A.A.P wrote the manuscript. All authors read and approved the manuscript.

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Conflict of Interest

The authors declare no Conflict of interest.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 9th Ed.Brussels, Belgium: *International Diabetes Federation*; 2019.
2. Saeedi P, Petersohn I, Salpea P, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157.
3. National Institute of Health Research and Development. Population Health Basic Health Research - RISKESDAS 2018. Jakarta, Indonesia: Ministry of Health Republic of Indonesia; 2019.
4. American Diabetes Association. Updates to the Standards of Medical Care in Diabetes-2018. *Diabetes Care.* 2018;41(9):2045-2047.
5. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. *Diabetologia*. 2001;44:S14-S21.
6. Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States. US *Department of Health and Human Services;* 2014..
7. Orozco-Beltran D, Gil-Guillen VF, Redon J, *et al*. Lipid profile, cardiovascular disease and mortality in a Mediterranean high-risk population: The ESCARVAL-RISK study. *PLoS One*. 2017;12(10).
8. Sartang MM, Mazloomi SM, Tanideh N, Zadeh AR. The effects of probiotic soymilk fortified with omega-3 on blood glucose, lipid profile, haematological and oxidative stress, and inflammatory parameters in

streptozotocin nicotinamide-induced diabetic rats. *J Diabetes Res*. 2015;2015.

9. Scientific concepts of functional foods in Europe. Consensus document. *Br J Nutr*. 1999;81 Suppl 1:S1-S27.
10. Coronel OADÁ, León-García E, Vela-Gutiérrez G, Medina JD la C, García-Varela R, García HS. Chayote (*Sechium edule* (Jacq.) Swartz). In: Fruit and Vegetable Phytochemicals. Vol 2. John Wiley & Sons, Ltd; 2017:979-992.
11. Loizzo MR, Bonesi M, Menichini F, Tenuta MC, Leporini M, Tundis R. Antioxidant and Carbohydrate-Hydrolysing Enzymes Potential of *Sechium edule* (Jacq.) Swartz (Cucurbitaceae) Peel, Leaves and Pulp Fresh and Processed. *Plant Foods Hum Nutr.* 2016;71(4):381-387.
12. Maity S, Firdous S, Debnath R. Evaluation of antidiabetic activity of ethanolic extract of *Sechium edule* fruits in alloxan-induced diabetic rats. *World J Pharm Pharm Sci*. 2013;2:3612-3621.
13. Neeraja K, Debnath R, Firdous SM. Cardioprotective activity of fruits of *Sechium edule*. *Bangladesh J Pharmacol.* 2015;10(1):125-130.
14. Tiwari AK, Reddy KS, Radhakrishnan J, *et al*. Influence of antioxidant rich fresh vegetable juices on starch induced postprandial hyperglycemia in rats. *Food Funct*. 2011;2(9):521-528.
15. Tiwari AK, Anusha I, Sumangali M, Kumar DA, Madhusudan K, Agawane SB. Preventive and Therapeutic Efficacies of Benincasa hispida and *Sechium edule* Fruit's Juice on Sweet-beverages Induced Impaired Glucose Tolerance and Oxidative Stress. *Pharmacologia.* 2013;4(3):197-207.
16. di Giuseppe D, Crippa A, Orsini N, Wolk A. Fish consumption and risk of rheumatoid arthritis: A dose-response meta-analysis. *Arthritis Res Ther.* 2014;16(1):1-7.
17. Lavigne C, Marette A, Jacques H. Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. *Am J Physiol - Endocrinol Metab.* 2000;278(3 41-3).
18. Ouellet V, Marois J, Weisnagel SJ, Jacques H. Dietary cod protein improves insulin sensitivity in insulin-resistant men and women: A randomized controlled trial. *Diabetes Care.* 2007;30(11):2816-2821.
19. Carroll KK. Biological effects of fish oils in relation toChronic diseases. *Lipids.* 1986;21(12):731-732.
20. Mehta JL, Lopez LM, Lawson D, Wargovich TJ, Williams LL. Dietary supplementation with omega-3 polyunsaturated fatty acids in patients with stable coronary heart disease. Effects on indices of platelet and neutrophil function and exercise performance. *Am J Med.* 1988;84(1):45-52.
21. Drotningsvik A, Mjos SA, Pampanin DM, *et al*. Dietary fish protein hydrolysates containing bioactive motifs affect serum and adipose tissue fatty acid compositions, serum lipids, postprandial glucose regulation and growth in obese Zucker fa/fa rats. *Br J Nutr.* 2016;116(8):1336-1345.
22. Hosomi R, Maeda H, Ikeda Y, Toda Y, Yoshida M, Fukunaga K. Differential effects of cod proteins and tuna proteins on serum and liver lipid profiles in rats fed Non-Cholesterol-And cholesterol-containing diets. *Prev Nutr Food Sci*. 2017;22(2):91-99.
23. AOAC. Official Methods of Analysis of AOAC International. 18th ed. (Horwitz W, Latimer GW, eds.). *AOAC International;* 2005.
24. Latimer G. Official methods of analysis of AOAC. Int 19th Ed AOAC Int Maryland, USA. Published online 2012.
25. Lobato LP, Iakmiu Camargo Pereira AE, Lazaretti MM, *et al*. Snack bars with high soy protein and isoflavone content for use in diets toControl dyslipidaemia. *Int J Food Sci Nutr.* 2012;63(1):49-58.
26. Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. *Exp Biol Med.* 2012;237(5):481-490.
27. Paget, G. E. and Barnes JM. Toxicity tests in evaluation of drug activities pharmacometries. In: Laurence DR, Bacharach AL, eds. Evaluation of Drug Activities. Academic Press; 1964.
28. Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. *Drug Des Devel Ther.* 2015;9:2515-2525.
29. Vieira EF, Pinho O, Ferreira IMPLVO, Delerue-

Matos C. Chayote (*Sechium edule*): A review of nutritional composition, bioactivities and potential applications. *Food Chem.* 2019;275:557-568.

30. Xia EQ, Zhu SS, He MJ, Luo F, Fu CZ, Zou T Bin. Marine Peptides as Potential Agents for the Management of Type 2 Diabetes Mellitus-A Prospect. *Mar Drugs.* 2017;15(4).
31. Stephen NM, Shakila JR, Jeyasekaran G, Sukumar D. Effect of different types of heat processing on chemical changes in tuna. *J Food Sci Technol.* 2010;47(2):174-181.
32. Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Adv Nutr.* 2012;3(1):1-7.
33. Calder PC. Mechanisms of Action of (n-3) Fatty Acids. J Nutr. 2012;142(3):592S-599S.
34. Khera A V., Cuchel M, de la Llera-Moya M, *et al*. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. *N Engl J Med*. 2011;364(2):127-135.
35. Tangka J, Banne Y, Dumanauw JM, Rumagit BI. The effect of tuna fish oil (*Thunnus s* albacares) on the total cholesterol, LDL cholesterol, HDL cholesterol and the triacylglycerol level on hypercholesterolemia rats (Rattus norvegicus). *Int J Pharm Clin Res.* 2016;8(5):Suppl: 451-456.
36. Markova M, Hornemann S, Sucher S, Pivovarova O, Pfeiffer A. Effects of an isocaloric high-protein diet in subjects with type 2 diabetes: Animal versus plant protein. *Exp Clin Endocrinol Diabetes.* 2015;122(03):P13_13.
37. Hamed I, Özogul F, Özogul Y, Regenstein JM. Marine Bioactive Compounds and Their Health Benefits: A Review. *Compr Rev Food Sci Food Saf.* 2015;14(4):446-465.
38. Pari L, Sankaranarayanan C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin-nicotinamide induced diabetic rats. *Life Sci.* 2009;85 (23-26):830-834.