**The Improvement of Lipid Profiles and Glucose Resistance of Hypercholesterolemic Rats using Tempeh Flour Based-Yoghurt Complemented with Red Pitaya Peel Extract**

Kartika Nugraheni1, Natalia Desy Putriningtyas2, G.S.N. Fernando3, Sunyoto Sunyoto4, Siti Harnina Bintari5

1Postgraduate Program of Nutrition, Universitas Muhammadiyah Semarang, Semarang City, Central Java, Indonesia

2 Department of Nutrition, Faculty of Medicine, Universitas Negeri Semarang, Semarang City, Central Java, Indonesia

3 Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka

4 Department of Mechanical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Semarang City, Central Java, Indonesia

5 Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang City, Central Java, Indonesia

Corresponding author: shbintari@mail.unnes.ac.id

**Abstract**

Tempeh flour is potentially a substitute material in making yoghurt that provides bioactive for improving cardiovascular health. It is also suitably combined with antioxidant sources, such as red pitaya peel extract, which is well known for inhibiting metabolic inflammation. This study aims to analyze the effectiveness of tempeh flour-based yoghurt complemented with red pitaya peel extract in improving lipid profiles and glucose tolerance in hypercholesterolemic rats. A total of 36 male Wistar rats were used and divided into six different groups: regular diet (ND) and five high-fat-diet rat groups treated with no supplementation (HFD); diary-based yoghurt with lactic acid bacteria (YBAL); commercial yoghurt (YB); tempeh flour based-yoghurt with lactic acid bacteria (SY); and tempeh flour based yoghurt complemented with red pitaya peel extract (SDS). The supplementation of each treatment group was given a dose of 3.6 ml/ KgBW/ day as a single dose for 28 days. The blood sample was collected from sinus orbitalis and then used for lipid profile, glucose, liver enzyme analysis, and statistical analysis using ANOVA. The result shows that glucose levels increased significantly in all groups, except HFD groups, and there is no significant difference between normal and yoghurt treatment groups. However, yoghurt and red pitaya peel extract supplementation significantly decrease LDL-C and increase HDL-C levels while improving liver condition during hypercholesterolemia. In conclusion, tempeh flour-based yoghurt complemented with red pitaya peel extract can improve lipid profile and liver health in hypercholesterolemia rats. Therefore, further research on metabolism pathways is needed to develop potential nutraceutical products for people with hypercholesterolemia.

**Keywords**: nutraceuticals, red pitaya, tempeh, yoghurt,

**Introduction**

Cardiovascular disease (CVD) is the leading cause of death, accounting for around a third of all deaths in Indonesia (M. A. Hussain et al., 2016). The projected number of people with cardiovascular disease in Indonesia is estimated to reach around six million in 2024 and continues to increase. The National Health Survey 2022 shows that more than 25% of Indonesian people have cardiovascular risk factors (Qanitha et al., 2022). The prevalence of cardiovascular disease has increased by up to 33% in men and women with smoking habits, high glucose and fat diets, and low physical activity (Adisasmito et al., 2020). Furthermore, cardiovascular disease is also increasing in the elderly population in Indonesia with high-risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and obesity (Zakaria et al., 2022). CVD is preceded by hypercholesterolemia, the lipid disorder condition characterized by high content of cholesterol, low-density lipoprotein-cholesterol (LDL-C), triglycerides and glucose and low high-density lipoprotein-cholesterol (HDL-C) content (Kaneko et al., 2021). Furthermore, a high-cholesterol diet increases LDL-C distribution and accumulation in the tissue triggers stress oxidation that growths become atherosclerosis (Fosbøl & Torp-Pedersen, 2019).

Several studies have shown that giving antioxidants from natural and fermented products reduces the risk of developing CVD in experimental animals with hypercholesterolemia (Olaniyan et al., 2017; Wihastuti et al., 2015). Consumption of antioxidants, especially polyphenols, can lower cholesterol and triglyceride levels, prevent lipid peroxidation, and protect blood vessels from free radicals (Ditano-Vázquez et al., 2019; Yamagata, 2019), as well as anti-inflammatory (Mofid et al., 2019). One of the best foods containing high polyphenol sources is tempeh, a fermented soybean seed using bacteria from the *Rhizopus* genus (Guo et al., 2023; Watanabe et al., 2023)*.* The soybean fermentation process enriches nutrient contents, protein digestibility, and essential amino acids, reducing the concentration of anti-nutritional compounds such as antitrypsin and phytic acid (Eklund-Jonsson et al., 2006). In addition, tempeh contains isoflavone compounds such as genistein, daidzen, and glycitein, which potentially reduce the risk of hypercholesterolemia and CVD (Yamagata & Yamori, 2021; Ye et al., 2021).

Tempe is a popular food commonly consumed by Indonesians; it is easy to get, cheap, and processable into derivative products such as yoghurt. Tempeh flour-based milk provides a suitable growth medium for lactic acid bacteria (LAB) that turn it into yoghurt (Bintari & Parman, 2019; Susanto et al., 2023). In addition, the content of LAB in the tempeh flour-based yoghurt provides pre- and probiotic agents for refining intestinal microflora and helps the digestive process. Tempeh flour-based yoghurt has a more extended expiration date than the original tempeh and can be developed as an alternative functional food product with high bioavailability (Bintari et al., 2017). The innovation of tempeh flour-based yoghurt was then complemented with other nutritional sources such as red pitaya fruit (*Hylocereus polyrhizus*) peel extract (RPPE). A previous study explains that supplementing a 25% mixture of RPPE in yoghurt reduces hypercholesterolemia risk in high-fat diet rats (Cahyati et al., 2021).

Red dragon fruit peel is usually discarded and considered as waste; in contrast, it has 33% of the total fruit weight (Song et al., 2016a) that contains betacyanin (which is identified as the primary red-violet pigment), pectin, triterpenoids, and steroids (Choo et al., 2018). The content of phenolic compounds, antioxidant properties, and dietary fibre in the peel is higher than in the flesh of red dragon fruit (Nurliyana et al., 2010). Kandungan nutrisi pada ekstrak kulit buah naga bermanfaat sebagai antiatherosclerosis (Nishikito et al., 2023), anti-diabetes (Panjaitan & Novitasari, 2021), and antiinflamasi (Joshi & Prabhakar, 2020) which can complement the nutritional value of tempeh yoghurt as a functional food without damaging the quality and taste (Madane et al., 2020). However, validation is needed to determine the ability to complement tempeh yoghurt with dragon fruit peel extract in conditions of hypercholesterolemia to reduce the risk of CVD. This study aims to analyze the effectiveness of tempeh yoghurt with the complementation of dragon fruit peel extract in improving lipid profiles and glucose tolerance in hypercholesterolemic conditions.

**Methods**

This experimental study is a pretest-posttest control group designed to determine the tempeh flour-based yoghurt complemented with RPPE administration's impact. The sample size used in this study follows the calculation of the Federer and Zelen (Federer & Zelen, 1966) formula, plus 20% of the total sample in each group in anticipation of dropping out due to death. Based on the calculation results, 36 three-month-old Wistar male rats with homogeneous body weight (190-200 g) were used in the study. The rats were then randomly divided into six treatment groups, as described in Table 1.

Table 1. Treatment groups

|  |  |
| --- | --- |
| Group  | Treatments  |
| ND | Not conditioned hypercholesterolemia |
| HFD | Hypercholesterolemic rats without treatment |
| YBAL | hypercholesterolemic rats; given as much BAL standard yoghurt |
| YB | hypercholesterolemic rats, topped with traditional commercial yoghurt |
| SY | hypercholesterolemic rats; given tempeh flour yoghurt |
| SDS | hypercholesterolemic rats; given tempeh flour yoghurt and dragon fruit skin |

Note: Single administration dosage of the treatment follows the substitute food recommendation for yoghurt for humans, as much as 200 ml/ KgBB/ day, then converted into rat dose following (Nair & Jacob, 2016). Based on the conversion, the treatment dose for rats is 3.6 ml/day and given to all groups.

**Tempeh flour-based yoghurt production**

The process of making yogurt begins with making yogurt starter. The process of making the starter begins with heating full cream milk to a temperature of 90oC and then cooling it down to 35oC. The next process is to add Lactobacillus bulgaricus or Streptococcus thermophilus 5% each of the amount of full cream milk and do the stirring process. The final stage of making yogurt starter is incubation in an incubator at 37-40oC for 18 hours.

Dragon fruit skin yogurt is produced using a ratio of 2:1 for full cream milk and dragon fruit skin extract. The mixture of full cream milk and dragon fruit peel extract is heated to a temperature of 90oC and then cooled to a temperature of 35oC. The next process is by adding each starter 5% Lactobacillus bulgaricus, Streptococcus thermophilus 5%, 10% sucrose and 1% tempeh flour. To make it more homogeneous, a stirring process is carried out for further incubation in the incubator. The mixture of ingredients was put into an incubator which was installed at 40oC for 8 hours.

**Red dragon fruit extraction and yoghurt production**

The ripe red pitaya fruit collected from the local market was washed under flowing water several times. The fruit skin was peeled and rewashed to remove the flesh, cut into small pieces and dried in an oven at 40°C for three days. The simplicia was crushed and macerated in 97% methanol for 24 hours. The solution was then homogenized and precipitated to separate the liquid and the dregs; then, the dregs were squeezed to extract the remaining solution and filtered using Whatman 41 paper until a clear solution was obtained. Methanol was evaporated using an evaporator at 70 °C until it stopped boiling. The red pitaya fruit peel extract was then dried in an oven at 40°C for five days. The obtained dry extract was ground until smooth and filtered using a 100-mesh sieve.

**Animals and Diet**

Animal facilities, management, and handling during the experiment were done in compliance with the Guidelines for Care and Use of Laboratory Animals of CNFS Universitas Gadjah Mada and approved by the Committee of Ethics and Health, Universitas Negeri Semarang (approval number: 140/KEPK/EC/2021). A total of 36 male rats of the albino Wistar strains (4 weeks old; 150-200 g) were supplied by the House of Experimental Rats, Center for Nutrition and Food Science (CNFS), Universitas Gadjah Mada, Yogyakarta, Indonesia. Environmental conditions such as 12:12 hours of light/dark cycle, the ambient temperature of 25.00 ± 1.00 °C, average humidity, and proper sanitation were maintained to minimise stress during the experiment. The rats were placed in individual stainless-steel cages. During the study, they were fed on a standard chow diet and given unrestricted access to water. The rats were also acclimatised for seven days before the initiation of the experiment.

**Hypercholesterolaemia induction and treatments.**

In all groups except ND, Hypercholesterolaemia was induced using a high-fat diet by administering 1% cholesterol for fourteen days. After that, the first observation was conducted on day 15 by measuring serum levels of total cholesterol, and then the hypercholesterolemic condition was determined when the cholesterol level was higher than 135.50 ± 15.64 mg/dl (Cunha et al., 2021).

Yoghurt treatment was administered orally by gastric intubation once daily for 28 days. The yoghurt was prepared according to research by Mardiana et al. (Mardiana et al., 2020). The rats' body weight was monitored weekly using an electric scale with an accuracy of 0.01 g. After 28 days of treatment, the rats were sacrificed by cervical decapitation. Blood samples were collected from all experimental rats pre- and post-treatment through sinus orbitalis, and then the carcases were burnt in an incinerator.

**Analysed Parameter**

Before and after the trial period, overnight fasting rats were profoundly anaesthetised. Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain the serum, which was then used to estimate the lipid profile. The mean levels of glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase (ALP) were determined by an automatic biochemistry analyser following the instructions of the manufacturer.

**Statistical Analysis**

The values are expressed as means ± standard deviation (SD) for six rats in each group. The significance of the difference between two sample means was subjected to the paired sample t-test. Differences between groups were assessed using the One-Way Analysis of Variance (ANOVA). If One-Way ANOVA yielded significant results, post hoc testing would be performed for intergroup comparisons using the least significant difference test. Values were considered statistically significant when p<0.05.

**RESULT AND DISCUSSION**

The observation showed a significant increase in body weight in hypercholesterolemic rats before and after yoghurt supplementation (Table 2). The highest increased body weight occurred in normal rats, while the lowest increase occurred in hypercholesterolemic rats (HFD) and those treated with standard commercial yoghurt (YB) (Table 2).

Table 2. The average body weight of the experimental animals during the study

|  |  |
| --- | --- |
| Groups | Body Weight (gr) |
|  | Pre (a) | Post (b) | ∆(b-a) | *p\** |
| ND | 197.60±5.27 | 233.00±4.53 | 35.40±1.14 | 0.001 |
| HFD | 204.40±4.67 | 228.60±5.41 | 24.20±1.30 | 0.001 |
| YBAL | 200.20±7.36 | 225.80±7.86 | 25.60±0.55 | 0.001 |
| YB | 199.80±4.92 | 224.20±4.32 | 24.40±1.67 | 0.001 |
| SY | 210.20±5.36 | 235.40±5.46 | 25.20±3.49 | 0.001 |
| SDS | 210.20±5.36 | 235.40±5.46 | 25.20±3.49 | 0.001 |

Note: *p\** indicates a significance level (p ≤ 0.05) between pre-and post-treatment analyzed using paired t-test; ANOVA statistical analysis showed no significant difference in body weight between groups at the 95% confidence level, with sig. level (p-value) ≤ 0.050. Pre: rat’s body weight before intervention; post: rat’s body weight after intervention; and ∆ = the score different between post and pretest.

The increase in rat’s body weight is not significantly different among groups; it may indicate that average growth in rats was unaffected by cholesterol administration and may related to the same feed application. The fed’s nutrient content, especially sugar, was considered normal to meet the rats' daily needs. This is because weight gain and obesity in hypercholesterolemic rats are triggered by exceeded intake of calory form carbohydrates that increase converting sugars into fatty acids stored in adipose as triglycerides (Guo et al., 2019).

The research results show that the blood sugar increased significantly in each group before and after the intervention (p<0.05). The rats’ average weight was supported by the pre- and post-treatment blood glucose levels, which decreased in the all yoghurt-treated group (Table 3). These conditions indicate the nutraceutical effect of the tempeh flour-based yoghurt complemented with RPPE in lowering blood glucose levels in hypercholesterolemic rats.

Table 3. Average blood glucose levels during the study

|  |  |
| --- | --- |
| Group | Blood glucose level (mg/dl) |
|  | Pre (a) | Post (b) | ∆(b-a) | *p* |
| ND | 63.39±1.18 | 64.72±1.50 | 1.33±0.44 | 0.437 |
| HFD | 161.92±3.18 | 164.11±3.82 | 2.19±1.07 | 0.000\* |
| YBAL | 158.70±4.17 | 144.15±1.10 | -14.55±4.46 | 0.001\* |
| YB | 166.04±4.91 | 147.72±2.29 | -18.33±3.31 | 0.001\* |
| SY | 158.48±5.65 | 109.88±3.05 | -48.60±6.02 | 0.001\* |
| SDS | 163.45±2.00 | 94.82±2.30 | -68.62±4.06 | 0.001\* |

Note: *\* =* indicates significance value (p<0.05) between pre- and posttreatment analyzed using paired t-test. Pre: rat’s body weight before intervention; post: rat’s body weight after intervention; and ∆ = the score different between post and pretest.

The decrease in blood glucose levels in hypercholesterolemic rats supplemented with yoghurt, tempeh flour-based yoghurt, and RPPE may be related to the role of nutrient content, which impacts glucose metabolism. For example, water-soluble fibre slows glucose digestion. It makes it absorbed gradually, giving a more prolonged satiety effect, and used for short-chain fatty acids (SCFA) synthesis by intestinal bacteria, thereby increasing insulin sensitivity (Afzal et al., 2020). Moreover, rat supplementation using tempeh flour-based yoghurt complemented with RPPEE may meet fibre needs that decrease rat’s appetite and reduce glucose intake. Metabolic fibre has low energy, so it probably does not contribute to gaining body weight (Yang et al., 2020). In addition, the polyphenol content in tempeh flour-based yoghurt, such as the flavonoid compounds, affects β-pancreas cell membrane permeability that triggers insulin release (Hussain et al., 2020). Another study also explained that rats supplemented with 3.4 ml of tempeh flour-based yoghurt dropped blood sugar levels up to 63.56% compared to the diabetic rat (Wahyu et al., 2022).

On the other side, several proteins from tempeh flour-based yoghurt are identified can control rat’s appetite by decreasing gastrointestinal hormone secretion, such as leptin and gastrin, reducing short-term food intake and diet-induced thermogenesis (Chungchunlam et al., 2015). The rich content of tryptophan and α-lactalbumin in tempeh flour-based yoghurt is absorbed quickly. It rapidly increases plasma amino acid concentration in the early 40 minutes to two hours after consumption before returning to basal level after 3-4 hours (Nongonierma & FitzGerald, 2015). Furthermore, tempeh flour-based yoghurt complemented RPPE administration and significantly improved lipid profiles by lowering triglyceride levels LDL-C while increasing HDL-C levels. Even so, measured total blood cholesterol levels in all yoghurt treatment groups were not significantly different than those in hypercholesterolemic rats (Figure 1).



Figure 1. Lipid profile in the control and treatment group rats given various types of yoghurt. The asterisk indicates the significance obtained from the one-way ANOVA test with a p-value <0.05 and a confidence level of 95%.

Based on the analysis, the rats treated with 3,6 ml of tempe flour-based yoghurt complemented with RPPE had the lowest LDL-C and triglyceride levels and higher HDL levels than the other treatment groups. A previous study found that 1.44 gr of the RPPE improves lipid profiles in male rats with dyslipidaemia (Werdiningsih, 2018). This result is in line with recent studies, which reported that the highest increase in HDL level was found in the treatment group having 800 mg/dl brewed dried pitaya fruit peel (Faadlilah & Ardiaria, 2016).

RPPE contains betalains compounds, such as betacyanins, bougainvillein-r-I, betanin, isobetanin, phyllocactin, isophyllocactin, and hylocerenin (Madadi et al., 2020). Several study states betacyanin, as a main bioactive content, contributes to total lipid regulation in the hepatic cells (Shen et al., 2019) by reducing lipid biosynthesis (Khoo et al., 2022) and increasing adiponectin serum levels while reducing fatty acid synthase (*Fas*) and fatty acid desaturase (*Fads2*) gene expression (Song et al., 2016b). This study showed no significant difference in the total cholesterol level in hypercholesterolemia conditions of all groups, including in tempeh flour-based yoghurt complemented with RPPE treatments. This indicates that tempeh yoghurt, whether supplemented with pitaya fruit peel extract or not, does not affect cholesterol absorption in rats. Meanwhile, decreased levels of LDL-C and increased HDL-C indicate that there may have been regulation of cholesterol transport in the liver induced by the bioactive compounds in red pitaya fruit extract. Although evidence has shown antihypercholesterolemic activity, the betalains mechanism in regulating lipid regulation has yet to be determined (Khoo et al., 2022).

The nutritional content in yoghurt, tempeh, red dragon fruit skin extract, or a combination of the three has been shown to help improve lipid profiles but not burden liver function. Based on the analysis results, the levels of liver enzymes such as SGPT, SGOT, and ALP decreased significantly in rats that were given red dragon fruit peel extract. The ability to maintain healthy liver function successively are tempeh yoghurt and red dragon fruit peel extract > tempeh yoghurt > commercial yoghurt > yoghurt with BAL standard (Figure 2).



Figure 2. Liver enzyme levels were measured from the blood of post-treated rats in each group of rats. The asterisk indicates the significance obtained from the one-way ANOVA test with a p-value <0.05 and a confidence level of 95%.

Hypercholesterolemia conditions can potentially burden liver function and trigger oxidative stress from cholesterol oxidation. However, based on serological analysis, administration of RPPE effectively reduced lipid accumulation and maintained liver health in hypercholesterolemia rats. This confirms that RPPE-complemented tempeh yoghurt can increase HDL-C levels, reduce serum SGPT, SGOT, and ALP levels, and relieve liver steatosis caused by HFD.

The content of bioactive compounds in tempeh yoghurt with RPPE complements, including genistein and daidzein from soybeans; flavonoids, niacin, and betalains from RPPE; as well as short-chain fatty acids (SCFA) from LAB possibly forming a synergetic effect that regulates lipid absorption and metabolism. LAB and daidzein increased the availability of T3 (thyroid hormone) in rat livers and decreased serum T4. Elevated T3 in the liver likely contributes to the activation of the cholesterol transport pathway to tissues (Šošić-Jurjević et al., 2019). In addition, genistein and daidzein trigger the activation of adenosine monophosphate-activated protein kinase (AMPK), a sensor protein in regulating cellular energy homeostasis (Tan et al., 2019a). Furthermore, these soy isoflavones can reduce the synthesis of the sterol regulatory element-binding protein family (SREBP-1) and inhibit the biosynthesis of lipids that consume ATP in adipocytes. In other words, soy isoflavones regulate adipocyte lipid metabolism through the AMPK/SREBP-1 pathway(Tan et al., 2019b).

The increasing HDL levels by RPPE yoghurt may be driven by flavonoid activity, which increases lecithin-cholesterol acyltransferase (LCAT) activity. LCAT converts free cholesterol into cholesterol ester that binds into lipoprotein core particles and forms HDL (Al-Muzaffar & Amin, 2017). Besides flavonoids, niacin content may also involve in lipid regulation. Moreover, niacin increases HDL-apoAI content level and HDL biogenesis by inhibiting the hepatocyte surface expression of β-chain ATP synthase and increasing the hepatic expression of ATP binding cassette subfamily A member 1 (ABCA1) (Naranjo et al., 2020). Also, niacin improves the physiological function of HDL by decreasing neutrophil myeloperoxidase (MPO) activity and cytokine-related macrophage inflammation (Hawkins & Davies, 2021). Niacin inhibits hepatocyte triglyceride synthesis and increases intracellular post-translational apoB and apoB-containing VLDL and LDL particles (Kamanna et al., 2013).

In conditions of hypercholesterolemia, cholesterol oxidation triggers MPO activation, resulting in increased production of hypochlorous acid (HOCl−), peroxynitrite (ONOO−), and cyanate (OCN−). These reactive oxidant compounds cause chlorination, nitration, carbamylation, and the formation of plasmalogen 2-chlorohexadecanal (2-ClHDA) oxidation products, which oxidize HDL (Marsche et al., 2022). Furthermore, HDL oxidation impairs the cholesterol transfer capacity via ABCA1. Still, the affinity for scavenger receptor class B type 1 (SR-BI) increases, thereby interfering with lipid uptake from the tissue into HDL (Gracia-Rubio et al., 2021). MPO oxidative modification of HDL results in the loss of the ability of HDL to activate endothelial nitric oxide synthase (eNOS) and inhibit cholesterol transport from macrophages (Marsche et al., 2022). Therefore, we suspect that oxidative scavenging activity involving niacin and other antioxidants from tempe yoghurt and red pitaya skin extract prevents oxidative stress and maintains the function of lipid transport through HDL.

Furthermore, red pitaya fruit peel contains several potentially bioactive compounds, including betalain, polyphenolic water-soluble pigment, and anthocyanin. The betacyanin pigment in red dragon fruit peel has recently been suggested as a primary betalain source (Hadipour et al., 2020; Liaotrakoon et al., 2013; Sadowska-Bartosz & Bartosz, 2021; Shen et al., 2019). At the same time, anthocyanins potentially inhibit cholesteryl ester transfer protein (CETP) activity, reducing the exchange of triglycerides from VLDL with cholesteryl esters from HDL and LDL. These conditions accelerate the cleansing process of free cholesterol excess to be delivered and removed in the liver; therefore, inflammation-triggered LDL oxidation was reduced (Nelson et al., 2022).

On the other hand, The composition of LAB in yoghurt also has the potential as a nutraceutical against hypercholesterolemia because it produces SCFAs, which play a role in the homeostasis of lipid metabolism. In adequate doses, probiotics in the fermented product (yoghurt and tempeh) can alter the intestinal microflora and act as an immunomodulator in the intestinal microhabitat (Pourrajab et al., 2020). The existence of probiotics and prebiotics not only acts as a stimulator of the growth and activity of probiotics but also has bio-functionality in improving gut health and immunity, lowering blood pressure and cholesterol levels, and even reducing the risk of inflammation (Mofid et al., 2019). The presence of LAB contributes to SCFA production, secondary bile acid reabsorption and lipopolysaccharide (LPS). SCFA is the product of non-digestible carbohydrate fermentation by gut bacteria and plays a role in activating receptors on enteroendocrine L cells to release GLP-1/GLP-2, which regulates postprandial chylomicron production (Yu et al., 2019).

Chylomicrons postprandial then interact with lipoprotein lipase (LPL) to hydrolyse triglycerides within it, releasing fatty acids and monoglycerides that can be taken up by tissues for energy production or storage (Cook et al., 2022). Furthermore, SCFAs are absorbed and enter the portal circulation towards the liver, act as substrates for de novo lipogenesis and contribute to VLDL production. Although giving yoghurt can increase SCFA levels, the breakdown of triglycerides for energy production does not occur due to the consistent intake of glucose from the feed. This condition may decrease triglyceride and blood cholesterol levels in hypercholesterolemic rats in this study. Other studies have also shown that probiotic yoghurt can significantly reduce total cholesterol and LDL-c in subjects with mild to moderate hypercholesterolemia without significantly affecting HDL-c and triglyceride levels.

## Conclusions

Combining tempe flour and red dragon fruit peel for yoghurt products improved lipid profile and inhibited liver cell damage related to oxidative stress in hypercholesterolemic Wistar rats. In addition, the combination of yoghurt, tempeh flour, and dragon fruit peel extract managed to reduce blood sugar levels and reduce the risk of obesity caused by HFD without affecting the rats' energy intake. Various pathways and molecular mechanisms will likely occur through synergetic and antagonistic interactions between bioactive compounds in tempeh yoghurt and red pitaya peel extract. Therefore, further research on the proteins and genes involved in cell signalling pathways is needed to elucidate more precise molecular physiological mechanisms. This can be developed to prepare safe and effective dietary and nutritional requirements for people with hypercholesterolemia.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Acknowledgements

The researchers acknowledge to Universitas Negeri Semarang for funding this research project.

## References

Adisasmito, W., Amir, V., Atin, A., Megraini, A., & Kusuma, D. (2020). Geographic and socioeconomic disparity in cardiovascular risk factors in Indonesia: analysis of the Basic Health Research 2018. *BMC Public Health*, *20*(1), 1004. https://doi.org/10.1186/s12889-020-09099-1

Afzal, U., Butt, M. S., Ashfaq, F., Bilal, A., & Suleria, H. A. R. (2020). Bioassessment of flaxseed powder and extract against hyperglycemia and hypercholesterolemia using Sprague Dawley rats. *Clinical Phytoscience*, *6*(1), 5. https://doi.org/10.1186/s40816-020-0150-y

Al-muzafar, H. M., & Amin, K. A. (2017). Efficacy of functional foods mixture in improving hypercholesterolemia, inflammatory and endothelial dysfunction biomarkers-induced by high cholesterol diet. *Lipid in Health and Disease*, *16*(194), 1–10. https://doi.org/10.1186/s12944-017-0585-4

Bintari, S. H., & Parman, S. (2019). Antioxidant capacity and nutritional value of tempe yoghurt. *Journal of Physics: Conference Series*, *1321*(3), 032048. https://doi.org/10.1088/1742-6596/1321/3/032048

Bintari, S. H., Widyastiti, N. S., Putriningtyas, N. D., Hapsari, R., & Nugraheni, K. (2017). Development and properties of tegurt, a yogurt-like tempe product. *Pakistan Journal of Nutrition*, *16*(4). https://doi.org/10.3923/pjn.2017.221.226

Cahyati, W. H., Siyam, N., & Putriningtyas, N. D. (2021). The potential of red dragon fruit peel yogurt to improve platelet levels in heparin-induced thrombocytopenia in Wistar rats. *Potravinarstvo Slovak Journal of Food Sciences*, *15*, 218–225. https://doi.org/10.5219/1497

Choo, K. Y., Kho, C., Ong, Y. Y., Thoo, Y. Y., Lim, R. L. H., Tan, C. P., & Ho, C. W. (2018). Studies on the storage stability of fermented red dragon fruit (Hylocereus polyrhizus) drink. *Food Science and Biotechnology*, *27*(5), 1411–1417. https://doi.org/10.1007/s10068-018-0367-4

Chungchunlam, S., Henare, S., Ganesh, S., & Moughan, P. (2015). Dietary whey protein influences plasma satiety-related hormones and plasma amino acids in normal-weight adult women. *Eur J Clin Nutr*, *69*, 179–186.

Cook, J. R., Kohan, A. B., & Haeusler, R. A. (2022). An Updated Perspective on the Dual-Track Model of Enterocyte Fat Metabolism. *Journal of Lipid Research*, *63*(11), 100278. https://doi.org/10.1016/j.jlr.2022.100278

Cunha, L. F., Ongaratto, M. A., Endres, M., & Barschak, A. G. (2021). Modelling hypercholesterolaemia in rats using high cholesterol diet. *International Journal of Experimental Pathology*, *102*(2), 74–79. https://doi.org/10.1111/iep.12387

Ditano-Vázquez, P., Torres-Peña, J. D., Galeano-Valle, F., Pérez-Caballero, A. I., Demelo-Rodríguez, P., Lopez-Miranda, J., Katsiki, N., Delgado-Lista, J., & Alvarez-Sala-Walther, L. A. (2019). The Fluid Aspect of the Mediterranean Diet in the Prevention and Management of Cardiovascular Disease and Diabetes: The Role of Polyphenol Content in Moderate Consumption of Wine and Olive Oil. *Nutrients*, *11*(11), 2833. https://doi.org/10.3390/nu11112833

Eklund-Jonsson, C., Sandberg, A. S., & Larsson Alminger, M. (2006). Reduction of phytate content while preserving minerals during whole grain cereal tempe fermentation. *Journal of Cereal Science*, *44*(2), 154–160. https://doi.org/10.1016/j.jcs.2006.05.005

Faadlilah, N., & Ardiaria, M. (2016). Efek Pemberian Seduhan Kulit Buah Naga Merah (Hylocereus polyrhizus) terhadap Kadar HDL Tikus Sprague Dawley Dislipidemia. *Journal of Nutrition College*, *5*(4), 280–288.

Federer, W. T., & Zelen, M. (1966). Analysis of multifactor classifications with unequal numbers of observations. *Biometrics*, *22*(3), 525–552.

Fosbøl, E., & Torp-pedersen, C. (2019). Ischaemic heart disease , infection , and treatment of infection. *European Heart Journal*, *0*, 1–2. https://doi.org/10.1093/eurheartj/ehz383

Gracia-Rubio, I., Martín, C., Civeira, F., & Cenarro, A. (2021). SR-B1, a Key Receptor Involved in the Progression of Cardiovascular Disease: A Perspective from Mice and Human Genetic Studies. *Biomedicines*, *9*(6), 612. https://doi.org/10.3390/biomedicines9060612

Guo, J., Li, K., Lin, Y., & Liu, Y. (2023). Protective effects and molecular mechanisms of tea polyphenols on cardiovascular diseases. *Frontiers in Nutrition*, *10*. https://doi.org/10.3389/fnut.2023.1202378

Guo, X. X., Zeng, Z., Qian, Y. Z., Qiu, J., Wang, K., Wang, Y., Ji, B.-P., & Zhou, F. (2019). Wheat Flour, Enriched with γ-Oryzanol, Phytosterol, and Ferulic Acid, Alleviates Lipid and Glucose Metabolism in High-Fat-Fructose-Fed Rats. *Nutrients*, *11*(7), 1697. https://doi.org/10.3390/nu11071697

Hadipour, E., Taleghani, A., Tayarani‐Najaran, N., & Tayarani‐Najaran, Z. (2020). Biological effects of red beetroot and betalains: A review. *Phytotherapy Research*, *34*(8), 1847–1867. https://doi.org/10.1002/ptr.6653

Hawkins, C. L., & Davies, M. J. (2021). Role of myeloperoxidase and oxidant formation in the extracellular environment in inflammation-induced tissue damage. *Free Radical Biology and Medicine*, *172*, 633–651. https://doi.org/10.1016/j.freeradbiomed.2021.07.007

Hussain, M. A., Al Mamun, A., Peters, S. A., Woodward, M., & Huxley, R. R. (2016). The Burden of Cardiovascular Disease Attributable to Major Modifiable Risk Factors in Indonesia. *Journal of Epidemiology*, *26*(10), 515–521. https://doi.org/10.2188/jea.JE20150178

Hussain, T., Tan, B., Murtaza, G., Liu, G., Rahu, N., Saleem Kalhoro, M., Hussain Kalhoro, D., Adebowale, T. O., Usman Mazhar, M., Rehman, Z. ur, Martínez, Y., Akber Khan, S., & Yin, Y. (2020). Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. *Pharmacological Research*, *152*, 104629. https://doi.org/10.1016/j.phrs.2020.104629

Joshi, M., & Prabhakar, B. (2020). Phytoconstituents and pharmaco-therapeutic benefits of pitaya : A wonder fruit. *Journal of Food Biochemistry*, *00*(e13260), 1–15. https://doi.org/10.1111/jfbc.13260

Kamanna, V. S., Ganji, S. H., & Kashyap, M. L. (2013). Recent advances in niacin and lipid metabolism. *Current Opinion in Lipidology*, *24*(3), 239–245. https://doi.org/10.1097/MOL.0b013e3283613a68

Kaneko, H., Itoh, H., Kiriyama, H., Kamon, T., Fujiu, K., Morita, K., Michihata, N., Jo, T., Takeda, N., Morita, H., Yasunaga, H., & Komuro, I. (2021). Lipid Profile and Subsequent Cardiovascular Disease among Young Adults Aged &lt; 50 Years. *The American Journal of Cardiology*, *142*, 59–65. https://doi.org/10.1016/j.amjcard.2020.11.038

Khoo, H. E., He, X., Tang, Y., Li, Z., Li, C., Zeng, Y., Tang, J., & Sun, J. (2022). Betacyanins and Anthocyanins in Pulp and Peel of Red Pitaya (Hylocereus polyrhizus cv. Jindu), Inhibition of Oxidative Stress, Lipid Reducing, and Cytotoxic Effects. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.894438

Liaotrakoon, W., de Clercq, N., van Hoed, V., van de Walle, D., Lewille, B., & Dewettinck, K. (2013). Impact of Thermal Treatment on Physicochemical, Antioxidative and Rheological Properties of White-Flesh and Red-Flesh Dragon Fruit (Hylocereus spp.) Purees. *Food and Bioprocess Technology*, *6*(2), 416–430. https://doi.org/10.1007/s11947-011-0722-4

Madadi, E., Mazloum-Ravasan, S., Yu, J. S., Ha, J. W., Hamishehkar, H., & Kim, K. H. (2020). Therapeutic Application of Betalains: A Review. *Plants*, *9*(9), 1219. https://doi.org/10.3390/plants9091219

Madane, P., Das, A. K., Nanda, P. K., Bandyopadhyay, S., Jagtap, P., Shewalkar, A., & Maity, B. (2020). Dragon fruit (Hylocereus undatus) peel as antioxidant dietary fibre on quality and lipid oxidation of chicken nuggets. *Journal of Food Science and Technology*, *57*(4), 1449–1461. https://doi.org/10.1007/s13197-019-04180-z

Mardiana, Budiono, I., & Putriningtyas, N. D. (2020). Comparison of organoleptic, protein, lipid and flavonoid content of commercial starter and isolated culture red dragon fruit peel yogurt. *Food Research*, *4*(3), 920–925. https://doi.org/https://doi.org/10.26656/fr.2017.4(3).380

Marsche, G., Stadler, J. T., Kargl, J., & Holzer, M. (2022). Understanding Myeloperoxidase-Induced Damage to HDL Structure and Function in the Vessel Wall: Implications for HDL-Based Therapies. *Antioxidants*, *11*(3), 556. https://doi.org/10.3390/antiox11030556

Mofid, V., Izadi, A., Mojtahedi, S. Y., & Khedmat, L. (2019). Therapeutic and Nutritional Effects of Synbiotic Yogurts in Children and Adults : a Clinical Review. *Probiotics and Antimicrobial Proteins*, 1–9. https://doi.org/10.1007/s12602-019-09594-x

Nair, A., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy*, *7*(2), 27. https://doi.org/10.4103/0976-0105.177703

Naranjo, M. C., Millan-Linares, M. C., & Montserrat-de la Paz, S. (2020). Niacin and hyperlipidemia. In *Molecular Nutrition* (pp. 263–281). Elsevier. https://doi.org/10.1016/B978-0-12-811907-5.00008-7

Nelson, A. J., Sniderman, A. D., Ditmarsch, M., Dicklin, M. R., Nicholls, S. J., Davidson, M. H., & Kastelein, J. J. P. (2022). Cholesteryl Ester Transfer Protein Inhibition Reduces Major Adverse Cardiovascular Events by Lowering Apolipoprotein B Levels. *International Journal of Molecular Sciences*, *23*(16), 9417. https://doi.org/10.3390/ijms23169417

Nishikito, D. F., Borges, A. C. A., Laurindo, L. F., Otoboni, A. M. M. B., Direito, R., Goulart, R. de A., Nicolau, C. C. T., Fiorini, A. M. R., Sinatora, R. V., & Barbalho, S. M. (2023). Anti-Inflammatory, Antioxidant, and Other Health Effects of Dragon Fruit and Potential Delivery Systems for Its Bioactive Compounds. *Pharmaceutics*, *15*(1), 159. https://doi.org/10.3390/pharmaceutics15010159

Nongonierma, A., & FitzGerald, R. (2015). Bioactive properties of milk proteins in humans: a review. *Peptides*, *73*, 20–34.

Nurliyana, R., Zahir, S., Suleiman, M., Aisyah, M., & Rahim, K. (2010). Antioxidant study of pulps and peels of dragon fruits : a comparative study. *International Food Research Journal*, *17*, 367–375.

Olaniyan, M., Ozuaruoke, D., & Afolabi, T. (2017). Cholesterol Lowering Effect of Cnidoscolous aconitifolius Leave Extracts in Egg Yolk Induced Hypercholesterolemia in Rabbit. *Journal of Advances in Medicine and Medical Research*, *23*(1), 1–6. https://doi.org/10.9734/jammr/2017/35431

Panjaitan, R. G. P., & Novitasari, N. (2021). Anti-diabetic Activity of the Red Dragon Fruit Peel (Hylocereus polyrhizus) in Ethanol Extract against Diabetic Rats. *Pharmacognosy Journal*, *13*(5), 1079–1085. https://doi.org/10.5530/pj.2021.13.140

Pourrajab, B., Fatahi, S., Dehnad, A., Kord Varkaneh, H., & Shidfar, F. (2020). The impact of probiotic yogurt consumption on lipid profiles in subjects with mild to moderate hypercholesterolemia: A systematic review and meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*, *30*(1), 11–22. https://doi.org/10.1016/j.numecd.2019.10.001

Qanitha, A., Qalby, N., Amir, M., Uiterwaal, C. S. P. M., Henriques, J. P. S., de Mol, B. A. J. M., & Mappangara, I. (2022). Clinical Cardiology in South East Asia: Indonesian Lessons from the Present towards Improvement. *Global Heart*, *17*(1). https://doi.org/10.5334/gh.1133

Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological Properties and Applications of Betalains. *Molecules*, *26*(9), 2520. https://doi.org/10.3390/molecules26092520

Shen, L., Xiong, X., Zhang, D., Zekrumah, M., Hu, Y., Gu, X., Wang, C., & Zou, X. (2019). Optimization of betacyanins from agricultural by‐products using pressurized hot water extraction for antioxidant and in vitro oleic acid‐induced steatohepatitis inhibitory activity. *Journal of Food Biochemistry*, *43*(12). https://doi.org/10.1111/jfbc.13044

Song, H., Chu, Q., Xu, D., Xu, Y., & Zheng, X. (2016a). Purified Betacyanins from Hylocereus undatus Peel Ameliorate Obesity and Insulin Resistance in High-Fat-Diet-Fed Mice. *Journal of Agricultural and Food Chemistry*, *64*(1), 236–244. https://doi.org/10.1021/acs.jafc.5b05177

Song, H., Chu, Q., Xu, D., Xu, Y., & Zheng, X. (2016b). Purified Betacyanins from Hylocereus undatus Peel Ameliorate Obesity and Insulin Resistance in High-Fat-Diet-Fed Mice. *Journal of Agricultural and Food Chemistry*, *64*(1), 236–244. https://doi.org/10.1021/acs.jafc.5b05177

Šošić-Jurjević, B., Lütjohann, D., Renko, K., Filipović, B., Radulović, N., Ajdžanović, V., Trifunović, S., Nestorović, N., Živanović, J., Manojlović Stojanoski, M., Kӧhrle, J., & Milošević, V. (2019). The isoflavones genistein and daidzein increase hepatic concentration of thyroid hormones and affect cholesterol metabolism in middle-aged male rats. *The Journal of Steroid Biochemistry and Molecular Biology*, *190*, 1–10. https://doi.org/10.1016/j.jsbmb.2019.03.009

Susanto, H., Ningrum, A. M., Noer, E. R., Muniroh, M., & Afifah, D. N. (2023). Differences effect of tempeh milk and tempeh yogurt on oxidative stress in maximal exercise. *Jurnal Aisyah : Jurnal Ilmu Kesehatan*, *8*(1), 39–42. https://doi.org/10.30604/jika.v8i1.1561

Tan, Huang, Luo, Liu, Cheng, Li, Xia, Li, Tang, Fang, Pan, Ou, Cheng, & Chen. (2019a). Soy Isoflavones Ameliorate Fatty Acid Metabolism of Visceral Adipose Tissue by Increasing the AMPK Activity in Male Rats with Diet-Induced Obesity (DIO). *Molecules*, *24*(15), 2809. https://doi.org/10.3390/molecules24152809

Tan, Huang, Luo, Liu, Cheng, Li, Xia, Li, Tang, Fang, Pan, Ou, Cheng, & Chen. (2019b). Soy Isoflavones Ameliorate Fatty Acid Metabolism of Visceral Adipose Tissue by Increasing the AMPK Activity in Male Rats with Diet-Induced Obesity (DIO). *Molecules*, *24*(15), 2809. https://doi.org/10.3390/molecules24152809

Wahyu, H. T., Anggi, V., Afrizal, A., Magfirah, M., & tandi, J. (2022). Potential Test of Soy-yamghurt against Antidiabetic in male white rats (Rattus norvegicus) Streptozotocin induced. *Research Journal of Pharmacy and Technology*, 4139–4143. https://doi.org/10.52711/0974-360X.2022.00695

Watanabe, N., Hara, Y., Tashiro, H., & Aoki, H. (2023). Antioxidant activity of tempe fermented with three different Rhizopus species. *Food Science and Technology Research*, *29*(2), FSTR-D-22-00167. https://doi.org/10.3136/fstr.FSTR-D-22-00167

Wiwik Werdiningsih, N., (2017). Efek Pemberian Kulit Buah Naga Merah (Hylocereus Polyrhizus) Terhadap Perubahan Profil Lipid Tikus Putih (Rattus Norvegicus) Jantan Yang Diberi Diet Tinggi Lemak (Doctoral Dissertation, Universitas Airlangga).

Wihastuti, T. A., Sargowo, D., Heriansyah, T., Aziza, Y. E., Puspitarini, D., Iwana, A. N., & Evitasari, L. A. (2015). The reduction of aorta histopathological images through inhibition of reactive oxygen species formation in hypercholesterolemia rattus norvegicus treated with polysaccharide peptide of Ganoderma lucidum. *Iranian Journal of Basic Medical Sciences*, *18*(5), 514–519. https://doi.org/10.22038/ijbms.2015.4416

Yamagata, K. (2019). Polyphenols Regulate Endothelial Functions and Reduce the Risk of Cardiovascular Disease. *Current Pharmaceutical Design*, *25*(22), 2443–2458. https://doi.org/10.2174/1381612825666190722100504

Yamagata, K., & Yamori, Y. (2021). Potential Effects of Soy Isoflavones on the Prevention of Metabolic Syndrome. *Molecules*, *26*(19), 5863. https://doi.org/10.3390/molecules26195863

Yang, J. H., Thu, T., Tran, T., & Le, V. V. M. (2020). *Hypolipidemic and Hepatoprotective Effects of High-Polydextrose Snack Food on Swiss Albino Mice*. *2020*.

Ye, Y.-B., He, K.-Y., Li, W.-L., Zhuo, S.-Y., Chen, Y.-M., Lu, W., Wu, S.-L., Liu, J., Li, Y.-B., & Zeng, F.-F. (2021). Effects of daidzein and genistein on markers of cardiovascular disease risk among women with impaired glucose regulation: a double-blind, randomized, placebo-controlled trial. *Food & Function*, *12*(17), 7997–8006. https://doi.org/10.1039/D1FO00712B

Yu, Y., Raka, F., & Adeli, K. (2019). The Role of the Gut Microbiota in Lipid and Lipoprotein Metabolism. *Journal of Clinical Medicine*, *8*(12), 2227. https://doi.org/10.3390/jcm8122227

Zakaria, S. I., Alfian, S. D., & Zakiyah, N. (2022). Determinants of Cardiovascular Diseases in the Elderly Population in Indonesia: Evidence from Population-Based Indonesian Family Life Survey (IFLS). *Vascular Health and Risk Management*, *Volume 18*, 905–914. https://doi.org/10.2147/VHRM.S390734