

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

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**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 analyse 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 was 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

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## INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death, accounting for around a third of all deaths in Indonesia (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 characterised 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 grows 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).

Tempeh 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; Bintari et al., 2022; 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., 2016) 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). The RPPE contains various nutrition that is beneficial for anti-atherosclerosis (Nishikito et al., 2023), anti-diabetes (Panjaitan & Novitasari, 2021), and anti-inflammation (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 analyse the effectiveness of tempeh yoghurt with the complementation of dragon fruit peel extract in improving lipid profiles and glucose tolerance in hypercholesterolemic conditions. This research is expected to provide new insight into developing processed tempe products and the use of unused fruit peel as a functional food and alternative products with nutritional and high selling value.

## 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.** Tempeh flour-based yoghurt and RPPE supplementation for hypercholesterolemic rat treatments.

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 yoghurt begins with making a yoghurt starter. Making the starter begins with heating full cream milk to a temperature of 90 °C and then cooling it to 35 °C. The following process was to add *Lactobacillus bulgaricus* or *Streptococcus thermophilus* 5% to each of the full cream milk and do the stirring process. The final stage of making a yoghurt starter was incubation at 37-40 °C for 18 hours.

Dragon fruit skin yoghurt was 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 was heated to a temperature of 90 °C and then cooled to 35 °C. The following process was conducted by adding each starter 5% *Lactobacillus bulgaricus* and 5% *Streptococcus thermophilus* with 10% sucrose and 1% tempeh flour. A stirring process was carried out to make it more homogeneous for further incubation. The mixture of ingredients was put into an incubator, which was installed at 40°C 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 homogenised 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.

### Hypercholesterolemia 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 carcasses were burnt in an incinerator.

### Analysed Parameter

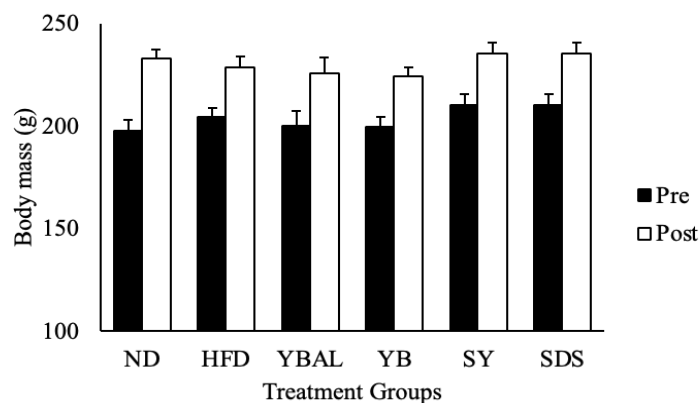
Before and after the trial period, overnight fasting rats were profoundly anaesthetised. Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain the serum, which was then used to estimate the lipid profile. The mean levels of glucose, total cholesterol (TC), low-density lipoprotein cholesterol (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  $\pm$  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 (Figure 1 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) (Figure 1).



**Figure 1.** The average body mass of the normal (ND) and hypercholesterolemic rats during pre- and post-supplementation of tempeh flour-based yoghurt and RPPE treatment. Note: An ANOVA statistical analysis showed no significant difference in body mass among groups at the 95% confidence level, with sig. level ( $p$ -value)  $\leq 0.050$ . Pre: rat's body mass before intervention; post: rat's body mass after intervention.

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 2). These conditions indicate the nutraceutical effect of the tempeh flour-based yoghurt complemented with RPPE in lowering blood glucose levels in hypercholesterolemic rats.

**Table 2.** Average hypercholesterolemic rat's blood glucose levels before and after tempeh flour-based yoghurt and RPPE supplementation.

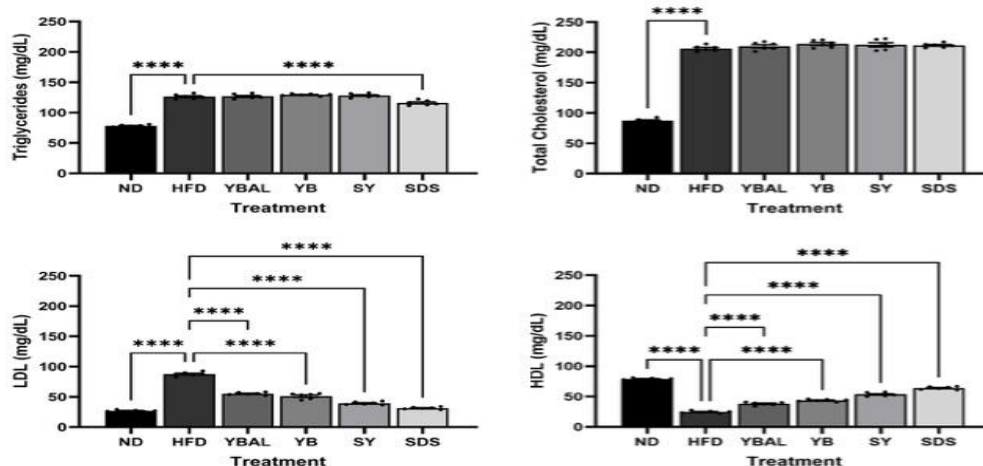
Group	Blood glucose level (mg/dl)			
	Pre	Post	$\Delta$	<i>p</i>
	(a)	(b)	(b-a)	
ND	63.39±1.18	64.72±1.50	1.33±0.44	0.437
HFD	161.92±3.18	164.11±3.82	2.19±1.07	0.000*
YBAL	158.70±4.17	144.15±1.10	-14.55±4.46	0.001*
YB	166.04±4.91	147.72±2.29	-18.33±3.31	0.001*
SY	158.48±5.65	109.88±3.05	-48.60±6.02	0.001*
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 analysed using paired t-test. Pre: rat's body weight before intervention; post: rat's body weight after intervention; and  $\Delta$  = 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 mass (Yang et al., 2020). In addition, the polyphenol content in tempeh flour-based yoghurt, such as the flavonoid compounds, affects  $\beta$ -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  $\alpha$ -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 2).

**Figure 2.** Lipid profile in the control and treatment group rats given various types of yoghurt.

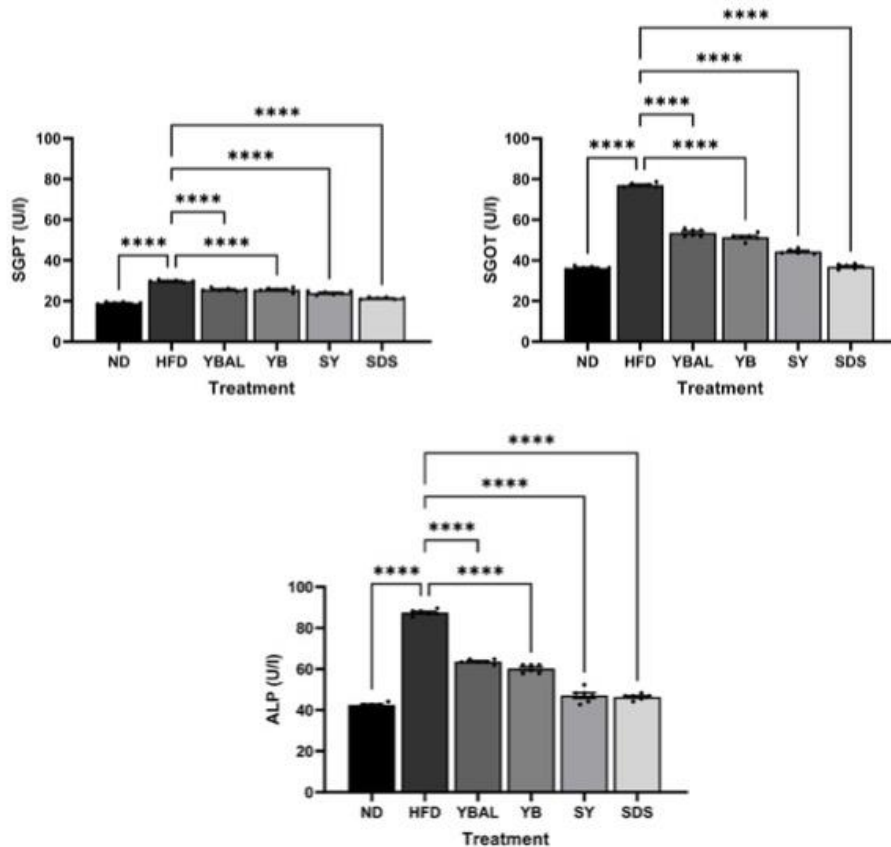
The asterisk indicates the significance obtained from the one-way ANOVA test with a  $p$ -value  $< 0.05$  and a confidence level of 95%.

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., 2016). 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 3).



**Figure 3.** 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., 2019). 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., 2019).

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  $\beta$ -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<sup>-</sup>), peroxyxynitrite (ONOO<sup>-</sup>), and cyanate (OCN<sup>-</sup>). These reactive oxidant compounds cause chlorination, nitration, carbamylation, and the formation of plasmalogen 2-chlorohexadecanal (2-ClHDA) oxidation products, which oxidise 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) (Rahayu & Qurbaniah, 2019). 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.



**Figure 4.** The ingredient of tempeh flour-based yoghurt consisted of tempeh flour and milk complementation with red pitaya peel extract.

This research proves that tempeh is a potential food source that can be processed into yoghurt without reducing its nutraceutical benefits. Furthermore, this research also shows that red pitaya/ dragon fruit peel, often discarded, can be used as a functional food source. Therefore, the combination of these two ingredients is potentially developed as an alternative diet for hypercholesterolemic or atherosclerosis sufferers. Further research should be conducted to determine the product's composition, public acceptance, and market potential as an innovative product with high economic value.

## CONCLUSIONS

Combining tempeh flour and red dragon fruit peel extract as a yoghurt product improves lipid profile by increasing HDL-serum and lowering

LDL-serum levels. Furthermore, tempeh flour-based yoghurt mixed with 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. This product also inhibits liver cell damage related to oxidative stress conditions caused by high glucose and cholesterol in hypercholesterolemic Wistar rats. In addition, the 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.



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