

Hypocholesterolemic Effects of Maysghurt as A Functional Food

Fatmawati Nur¹, Hafsan^{1*}, Muh. Khalifah Mustami²,Khaerani Kiramang³, Aminah Hajah Thaha⁴

^{1,2}Department of Biology, Universitas Islam Negeri Alauddin, Indonesia

^{3,4}Department of Animal Science, Universitas Islam Negeri Alauddin, Indonesia

Abstract

Hypercholesterolemia is known to have a strong correlation with cardiovascular disease cases. One alternative action that can reduce cholesterol levels in the blood is the provision of functional food. Not many studies have revealed the functional effects of maysghurt, a fermented drink made from sweet corn, to treat hypercholesterolemia. This experiment consisted of six treatment groups to determine the effect of hypercholesterolemia therapy with maysghurt on white male rats Rattus norvegicus Wistar strain in terms of serum and digesta total cholesterol levels, HDL, LDL and the ratio of LDL to HDL. This study used 46 male Rattus norvegicus. Rats models of hypercholesterolemia were obtained by giving Hyper cholesterol-inducing suspension (HIS) in the form of the beef brain that had been steamed and blended at a dose of 2 mL/day. The therapy of giving maysphurt was through gastric sonde technique with doses of 2 mL, 3 mL, 4 mL and 5 mL per 200 g of body weight of rats. The analysis used in this study is the One Way Analysis of Variance with Duncan's 5% further test. The results of the data analysis can be concluded that the gave maysghurt has a significant effect on reducing total blood cholesterol and digesta levels, and there is a positive correlation between serum and digesta cholesterol levels-the lower the serum cholesterol, the lower the digesta cholesterol of the rats. Maysghurt therapy reduced LDL levels and significantly increased HDL levels in hypercholesterolemic Wistar strain rats (Rattus norvegicus). This study also showed that maysghurt at a dose of 3 mL/200 g body weight of rats was the most effective in increasing HDL and lowering LDL in hypercholesterolemic rats. In general, this study reveals that maysghurt can reduce LDL levels, increase HDL levels, and improve the ratio of LDL to HDL so that it has the potential to reduce the risk of cardiovascular disease.

Keywords: cholesterol, HDL, hypercholesterolemia, maysghurt, LDL

Introduction :

Cardiovascular disease, a condition of disorders of the heart and blood vessels, is the leading cause of death globally (Walker, 2013). According to findings in the last 30 years, the disease is the cause of a third of all deaths globally, and the number of deaths continues to increase (Kjeldsen et al., 2021; Félix-Redondo et al., 2013). With age, both women and men can experience the disease as a risk factor (Carson et al., 2020). Many studies have revealed a correlation between the occurrence of cardiovascular disease and abnormal blood cholesterol levels, namely high cholesterol levels that exceed normal limits (Soliman, 2018; Hansel & Giral, 2015; Walker, 2013). This condition is known as hypercholesterolemia (Kundu et al., 2021; Di Taranto et al., 2019). Increased blood cholesterol levels are influenced by several risk factors, including genetics, age, gender, body mass index, and lifestyle closely related to the foods consumed (Jesch & Carr, 2017; Kim et al., 2017; Félix-Redondo et al., 2013). Foods such as meat, liver, brain, and offal given to experimental animals have been shown to cause excess cholesterol in the blood (Lafuente & De las Heras, 2018; Sarita, 2012). The state of hypercholesterolemia in animals occurs when the total cholesterol level in the blood exceeds normal. Elevated cholesterol levels can lead to narrowing blood vessels or atherosclerosis (Aguilar-Ballester et al., 2020; Kucuk et al., 2017). To avoid this condition, proper control of blood cholesterol levels is needed. One of the safe ways to lower blood cholesterol levels is by modifying the diet. In general, the recommended diet limits the consumption of foods that contain cholesterol and fat, exceptionally high saturated fat (Walker, 2013). This diet provides a safer effect, so it is highly recommended before deciding on drug therapy. In addition to limiting

the consumption of cholesterol and fat, it is also recommended to take anti-hypercholesterolemia (Andriani et al., 2020; Cheng et al., 2018). In general, the use of anti-hypercholesterolemic drugs, such as the class of hydroxy-3 methyl glutaric-coenzyme A (HMG Co-A) reductase inhibitors and drugs that can increase bile acid excretion, has been successful in controlling and lowering blood cholesterol levels. However, long-term use of hypercholesterolemic drugs will cause side effects such as anxiety and can affect liver function (Caponio et al., 2020; Y. Hu et al., 2020), so that functional foods can be an option for controlling hypercholesterolemia (Alongi & Anese, 2021; Birch & Bonwick, 2019).

Maysghurt is a processed product of corn juice fermented by lactic acid bacteria, namely *Lactobacillus fermentum* (Supavititpatana et al., 2010; Trikoomdun & Leenanon, 2016). Based on the advantages of lactic acid bacteria in fermenting corn milk (cider), these isolates can quickly adapt to their substrates and remodel complex compounds into simpler compounds to produce lactic acid in the product. Lactic acid bacteria used as fermenters are expected to be able to decompose lactose into lactic acid, which will synergistically bind cholesterol in the small intestine through its cell walls before the body absorbs cholesterol (Widodo et al., 2021; Aloğlu & Öner, 2006; Pereira & Gibson, 2002) or reduce cholesterol levels by some other mechanism.

Probiotics in maysghurt can produce the enzyme Bile Salt Hydrolase (BSH), an enzyme that can reduce bile salt conjugation (Kingkaew & Tanasupawat, 2019; Pavlović et al., 2012; Liong & Shah, 2005). This will increase the levels of free bile acids, which are not easily absorbed by the small intestine, compared to bile acids (Adebola et al., 2020; Pavlović et al., 2012). Efforts to balance the number of bile acids in the body take cholesterol taken from the blood, which functions as a precursor, so that cholesterol levels can be lowered in total (Choi et al., 2015; Baila-Rueda et al., 2014). Other cholesterol degradation pathways can also occur through the conversion of cholesterol to cholic bile acid by lactic acid bacteria in maysghurt so that the concentration of cholesterol in the blood can be reduced and cholesterol levels become more stable (Horáčková et al., 2018; Gérard, 2013; Chiang, 1998).

Several epidemiological and clinical studies have shown that total plasma cholesterol and Low-Density Lipoprotein (LDL) are major risk factors for heart attack (Deng, 2009a). This study revealed the potential of maysghurt for animal therapy models of hypercholesterolemia in terms of High-Density Lipoprotein (HDL) and LDL serum and LDL/HDL ratio, which are the best predictors of coronary heart disease risk compared to LDL cholesterol or HDL cholesterol only (Di Taranto et al., 2019; Kucuk et al., 2017; Kunutsor et al., 2017). In addition, digesta cholesterol levels were also measured to assess the description of the mechanism of cholesterol reduction that occurred (Welli et al., 2019).

The negative effect of cholesterol-lowering drugs triggers increased public awareness to consume safe and healthy functional foods (Mitsuoka, 2014; Pastrana et al., 2017). This encourages fermented products known to be safe and has advantages in nutritional and health aspects (Alongi & Anese, 2021; Birch & Bonwick, 2019; Annunziata & Vecchio, 2013; Shah, 2007). Equally important is the opportunity to explore various functional foods such as fermented milk that can be modified based on their raw materials. The use of sweet corn as raw material for fermented milk to replace cow's milk can support food security and diversify food products from corn (Karneta, 2019; Trikoomdun & Leenanon, 2016).

Methods :

This experiment consisted of six treatment groups for hypercholesterolemia therapy with maysghurt on white male rats (*Rattus norvegicus*) Wistar strain. The male rats used were two months old with relatively average weight obtained from the test animal development unit. The

experimental rats were first acclimatized for one week by being housed with a 12-hour lighting cycle (Saikia et al., 2018; Yadav et al., 2007). Standard rodent feed and drinking water have been provided ad libithum. The number of research subjects used seven rats each for six treatment groups, as stipulated by WHO, which requires a minimum number of subjects per group, a minimum of five individuals.

For 21 days after acclimation and initial weighing of the rats, the other five groups were given a high-cholesterol diet apart from the control group. Four of them were given different doses of maysghurt. The hyper cholesterol-inducing suspension (HIS) given was in the form of the beef brain steamed and blended at a dose of 2 mL/day (Kundu et al., 2021). The dose of maysghurt is based on the recommended dose for fermented milk for humans weighing 70 kg, which is approximately 100-200 mL/day (Trikoomdun & Leenanon, 2016). This dose was then converted to a dose for rats weighing 200 g so that the doses of 2 mL, 3 mL, 4 mL and 5 mL per 200 g body weight of rats were obtained. The administration of hypercholesterolemia-inducing suspension and maysghurt was carried out using a gastric sonde technique (Singh et al., 2015).

The maysghurt used is fermented milk made from corn milk with 107 CFU/mL (Trikoomdun & Leenanon, 2016)(Karneta, 2019). The preparation of maysghurt begins with starter preparation by multiplying *L. Fermentum* pure cultures by transferring them into several test tubes containing sterile De Man Rogosa and Sharpe (MRS) media. Furthermore, 25% w/v skim milk and 5% w/v sugar were pasteurized at 90°C for 15 minutes and then cooled to 40°C; 5 ml was mixed with culture and incubated at 40°C for 1 x 24 hours. Corn milk was prepared by mashing as much as 700 grams of corn using a blender with a ratio of sweet corn kernels: water is 1: 5 and then filtered to get sweet corn juice. The corn extract was added with 5% w/v skimmed milk powder, pasteurized at 90°C for 15 minutes, and then cooled to 40°C.

Furthermore, as much as 10% *L. fermentum* starter was inoculated little by a little while stirring. It was homogeneous and put into prepared sterile containers, then covered with aluminium foil. Then it was incubated in an incubator at 45oC for 7 hours to produce maysghurt with an acidic pH of 4-5 (Trikoomdun & Leenanon, 2016)(Karneta, 2019). After the incubation is complete, the fermented milk produced is immediately cooled in the refrigerator so that the fermentation does not continue. Before being applied to experimental rats, a proximate maysghurt analysis was first carried out, which refers to (AOAC, 2005), including fat content, protein content, total sugar content, colour, taste, aroma, and texture. Observations were made after the inactivation of the fermentation process, namely after being put in the refrigerator for 2 hours.

Each treatment group measured the total blood cholesterol levels of LDL, HDL, and LDL/HDL ratio at the end of the treatment, namely on day 21. Bodyweight was first measured using a particular scale for experimental rats, namely the Triple Beam Balance. Blood samples were taken from the pre-orbital plexus of rats at the edge of the right eye and put into a hematocrit capillary pipette. 150-250 L was collected in an Eppendorf tube and then centrifuged for 15 minutes at 3000 rpm to obtain the serum. Total cholesterol, HDL, and LDL cholesterol levels in rat blood were examined by an enzymatic spectrophotometric method using CHO-PAP and GPO-PAP (glycerol-3-phosphate peroxidase amino antipyrine phenol) (Friedewald et al., 1972). The LDL/HDL ratio is determined by comparing LDL levels to HDL levels. Digesta cholesterol taken from the cecum of the treated rats was measured at the end of the treatment. The data obtained were processed by computer applications. The data were tested for normality with the Shapiro Wilks test. Differences in serum total, LDL and HDL cholesterol levels, as well as LDL/HDL ratio and digesta cholesterol levels in each treatment group, were analyzed to determine the effect of the six groups using the Anova parametric statistical test, followed by Duncan's test at a 95% confidence level. The procedure in

this research has been approved by the Health Research Ethics Commission of UIN Alauddin Makassar based on ethical clearance number 078/KEPK/IX/2020.

Result and Discussion :

Physicochemical characteristics of maysghurt as a functional food :

Maysghurt as a fermented milk product projected as a functional food has physicochemical characteristics identified as in table 1. The relatively low fat and carbohydrate content accompanied by high protein content and the content of lactic acid bacteria cells in maysghurt can be beneficial if consumed. (F. B. Hu, 2005). This is not only because the content is a nutrient that the body needs. It can also have a positive physiological effect accompanied by live microbes, namely lactic acid bacteria (Bernal Castro et al., 2017; Kanekanian, 2014). Pastrana et al. (2017) describe that functional food is food that, due to its active component content, can provide health benefits beyond the benefits provided by the nutrients contained in it and fulfils sensory, nutritional and physiological requirements.

From the sensory aspect, the colour, taste and aroma of maysghurt show an attractive appearance with a delicious taste. The yellow colour of maysghurt is caused by the primary colour of corn milk, which is slightly yellowish due to the carotenoid content contained in corn and becomes more concentrated due to the work of inoculant bacteria that produce lactic acid so that the yellow colour formed is more concentrated (Supavititpatana et al., 2010; Brookfield, 2009;).

No	Analysis	Description	
1	Total number of BAL	7.8 log (CFU/mL)	
2	Water content	80.79 %	
3	Ash Level	0.87 %	
4	Fat level	3.12 %	
5	Protein Level	11.88 %	
6	Total Carbohydrate Level	3.34 %	
7	рН	4.09	
8	Consistency	thick	
9	Scent	distinctive scent (sour)	
10	Flavour	sour	
11	Colour	yellow	

Table 1. Physicochemical characteristics of maysghurt

The distinctive scent of maysghurt is like a sour scent, as well as a sour taste. This sour aroma and taste arise because there is a change in milk lactose into lactic acid by lactic acid bacteria (Pramono et al., 2020). The sour maysghurt aroma still leaves the corn milk scent. In addition, the distinctive scent of maysghurt is strongly influenced by the scent-forming compounds produced by lactose during fermentation, such as diacetyl. Diacetyl is the main flavour component in fermented milk products synthesized by starters that can metabolize citrate (Astawan et al., 2012; Supavititpatana et al., 2010).

The texture of the resulting product will undoubtedly affect the consumer's acceptance of the product (Rahayu & Andriani, 2018; Costa et al., 2017; Trikoomdun & Leenanon, 2016; Janzantti et al., 2011). Good yoghurt is neither too thick nor too liquid and has texture stability without decreasing viscosity during storage. Lactic acid bacteria will trigger the fermentation process in corn milk, then convert the lactose in corn milk into lactic acid, which gives the effect of breaking

the corn milk protein, which causes the corn milk to thicken, so the maysghurt tastes sour and has a thick texture (Pramono et al., 2020; Rahayu & Andriani, 2018).

The live weight of the experimental rat :

During the experiment, the live weight of rats for all treatment groups increased. Among all treatments, there was a very significant difference in body weight gain of rats. The graphs of the bodyweight of rats before and after treatment for each treatment group are described in Figure 1. Rats with standard feeding treatment and induced with hyper cholesterol-inducing suspension without maysghurt showed the highest body weight gain. The treatment group that was given a standard diet plus a hyper cholesterol suspension and supplemented with maysghurt in general also showed a significant difference in body weight gain compared to the group of rats that were only given standard feed (T0). This can be explained that the beef brain is known to have a relatively high nutritional content, apart from cholesterol (Piaggi, 2019; Joyce et al., 2014). It is known that in 100 grams of the beef brain, there are 150 calories. In addition, there are 3100 milligrams of cholesterol, 108 milligrams of sodium, 224 milligrams of potassium, 1.5 grams of carbohydrates and 12 grams of protein (Kundu et al., 2021; Vedaraman et al., 2005). The content of these nutrients supports the formation of cells in the body so that muscle mass can develop and grow properly (Joyce et al., 2014).

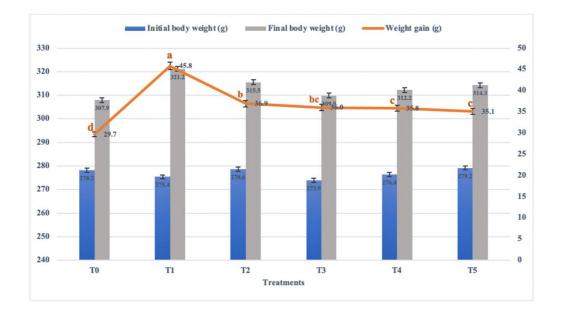


Figure 1. Body weight of rats before and after treatment (T0 = standard feed; T1 = standard feed + HIS; T2 = standard feed + HIS + 2 mL maysghurt; T3 = standard feed + HIS + 3 mL maysghurt; T4 = standard feed + HIS + 4 mL maysghurt; T5 = standard feed + HIS + 5 mL maysghurt) and the increase in body weight of rats after treatment. The different letter notations represent significant differences (P < 0.05). Maysghurt supplementation indicated a significant suppression effect on body weight gain in rats (P < 0.05), even when given a hyper cholesterol-inducing diet. The trend is shown in Figure 1, which shows a decrease in the T2, T3, T4 and T5 treatment groups. Maysghurt's ability to reduce weight is related to its protein content. Some researchers explain that there are three main mechanisms related to the role of protein in weight loss, namely increasing satiety, increasing thermogenesis (increased energy expenditure above metabolic rate), which also has an impact on satiety (Leidy et al., 2015; Hu, 2005). The decrease was relatively the same at the maysghurt concentration of 3-5 mL. This implies that a concentration of 3 mL/200-

gram body weight of rats is sufficient to maintain the body weight of rats. A higher volume of maysghurt will not impact reducing or increasing the bodyweight of rats.

Hypocholesterolemic effect of maysghurt :

Pemberian penginduksi hiperkolesterol selama 21 hari berupa suspensi otak sapi terbukti dapat meningkatkan kadar kolesterol serum darah tikus putih (*Rattus norvegicus*). This finding follows Kundu et al. (2021), and Vedaraman et al. (2005) explained that rats' total cholesterol, triglyceride, and LDL blood levels increased significantly after consuming beef brain. Figure 1 describes the blood cholesterol and digesta levels of rats induced by hypercholesterolemia compared to rats fed an only standard diet with and without maysghurt supplementation. The increase in blood cholesterol levels in white rats was due to the high cholesterol content of beef brain suspension, so that the absorption of cholesterol in the intestines increased. Increased absorption of cholesterol levels in the blood of the white rat (*Rattus norvegicus*) (Kundu et al., 2021; Vedaraman et al., 2005). Figure 1 also shows a decrease in cholesterol levels both in the blood and in the digesta of rats in the treatment of cow brain suspension supplemented with maysghurt at different concentrations. This is consistent with the results of studies conducted using various fermented milk (Bhat et al., 2019; Sengupta et al., 2019; Kanekanian, 2014; Astawan et al., 2012; Ramchandran & Shah, 2011Deng, 2009; Sarkar, 2008).

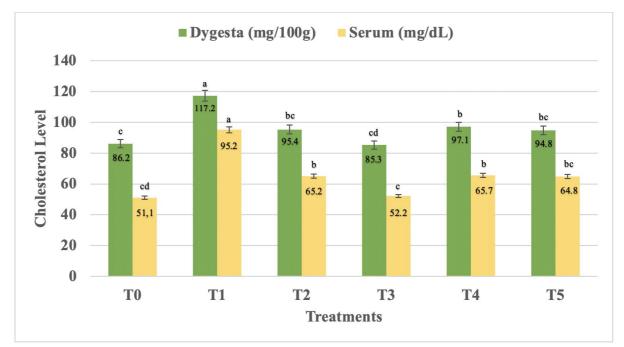


Figure 2. Total serum and digesta cholesterol levels after treatment (T0 = standard feed; T1 = standard feed + HIS; T2 = standard feed + HIS + 2 mL maysghurt; T3 = standard feed + HIS + 3 mL maysghurt; T4 = standard feed + HIS + 4 mL maysghurt; T5 = standard feed + HIS + 5 mL maysghurt) and the increase in body weight of rats after treatment. The different letter notations represent significant differences (P < 0.05). The graph in figure 2 shows a significant difference between serum and digesta cholesterol levels in each treatment. A positive correlation also occurs between serum and digesta cholesterol levels—the lower the serum cholesterol, the lower the digesta cholesterol of the rats.

According to Kingkaew & Tanasupawat (2019), Cholesterol absorption depends on the availability of bile acids from the liver and pancreatic cholesterol esterase and the amount of cholesterol

intake. Increasing cholesterol intake can reduce cholesterol absorption (MacDonald et al., 2020; Xu et al., 2018). At high cholesterol concentrations, the body will absorb less than 10% cholesterol, and the rest will leave the body through faeces (Choi et al., 2015; Gérard, 2013). Control of cholesterol synthesis through cholesterol intake is crucial because when cholesterol intake is high, cholesterol synthesis decreases and vice versa (Kim et al., 2017; Baila-Rueda et al., 2014).

Based on the analysis of digesta cholesterol levels, it can be explained that the mechanism of cholesterol reduction with maysphurt supplementation may occur due to the bile salt deconjugation process due to the activity of the bile salt hydrolase (BSH) enzyme produced by inoculant bacteria, namely Lactobacillus fermentum. With the presence of the BSH enzyme in the digestive tract, the work of HMG-CoA may be inhibited in producing cholesterol (Khare & Gaur, 2020; Nuhwa et al., 2019; Singh et al., 2015). The mechanism of the ability of the BSH enzyme to work in vivo results in the inhibition of the HMG-CoA reductase enzyme, which plays a role in the formation of mevalonate in the cholesterol synthesis process does not form cholesterol (Joyce et al., 2014). According to Liu et al. (2021), the decrease in cholesterol occurred because the compounds produced by microbes competed with HMG-CoA to bind to the HMG-CoA reductase enzyme. BSH produced by LAB plays a role in forming deconjugated bile acids by removing water molecules between glycine and cholic acid to produce unconjugated bile acid. Free cholic acid is less readily absorbed in the small intestine than bile acids bound to glycine. Deconjugated bile acids (free cholic acid) will be wasted through the faeces so that the amount of bile acids that return to the liver is more diminutive. In this condition, the need for cholesterol increases, and consequently, cholesterol levels in the blood decrease. To balance the number of bile acids, the body will take body cholesterol as a precursor. This process, in turn, lowers overall blood cholesterol levels. The mechanism of inhibition of the BSH enzyme on the synthesis of conjugated bile acids is molecular, and the enzyme breaks the C24NaCl amide peptide bond between bile salts and amino acids. So that glycine or taurine loses its hydroxyl group and results in the formation of free cholic acid (Kingkaew & Tanasupawat, 2019; Choi et al., 2015; R. Kumar et al., 2012; Pavlović et al., 2012; Liong & Shah, 2005; Moser & Savage, 2001).

The findings of this study are in line with the findings of (Shiby & Mishra, 2013; Oh et al., 2012), who has investigated the hypocholesterolemic effect of *L. acidophilus* from fermented milk in rats that the ability to deconjugate bile salts is more dominant than the ability to assimilate cholesterol. In the body, the ability to assimilate cholesterol is bound to cholesterol from outside the body (feed cholesterol). In contrast, the deconjugated bile salts, which are closely related to cholesterol, are cholesterol synthesized by the body.

The description of Figure 2 also shows that the concentration of 3 mL/200-gram weight of rats effectively reduces the blood serum cholesterol levels of rats. This means that the number of lactic acid bacteria cells at that concentration has effectively carried out the bile deconjugation function to suppress the formation of cholesterol. This decrease is by the results of research to prove the advantages of lactic acid bacteria given in the form of fermented milk, namely yoghurt containing these bacterial cells. The results of this study indicate that lactic acid bacteria can cause a decrease in blood serum cholesterol in experimental animals (Kingkaew & Tanasupawat, 2019; M. Kumar et al., 2012).

The relatively high levels of cholesterol in the digesta in this study have not been able to explain the mechanism of reducing blood cholesterol levels through the assimilation/ utilization of cholesterol by lactic acid bacteria, as revealed by (Widodo et al., 2021). The cholesterol assimilation occurs through the mechanism of cholesterol uptake by Lactic Acid Bacteria, which then incorporates the cholesterol into the bacterial cell membrane, causing a reduction in the amount of free cholesterol in the body of the test animal. (Ishimwe et al., 2015; M. Kumar et al., 2012).

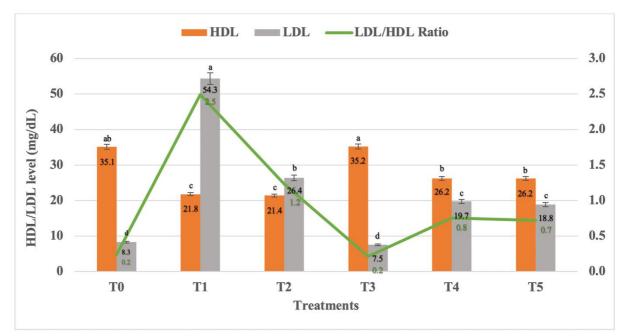


Figure 3. HDL and LDL levels, LDL/HDL ratio after treatment (T0 = standard feed; T1 = standard feed + HIS; T2 = standard feed + HIS + 2 mL maysghurt; T3 = standard feed + HIS + 3 mL maysghurt; T4 = standard feed + HIS + 4 mL maysghurt; T5 = standard feed + HIS + 5 mL maysghurt) and the increase in body weight of rats after treatment. The different letter notations represent significant differences (P < 0.05). Figure 3 shows a decrease in LDL in all treatments after the administration of maysghurt for 21 days, and the most significant decrease occurred at a dose of 4 mL. Likewise, based on statistical tests, it can be seen that the administration of maysphurt at all treatment doses was able to increase HDL cholesterol levels and reduce LDL/HDL ratios. The occurrence of an increase in LDL and a decrease in HDL in the hypercholesterolemic state without giving maysghurt is due to the accumulation of cholesterol in the blood due to the induction of hypercholesterolemia. An increase in LDL levels and a decrease in HDL levels is due to excess cholesterol, which causes a build-up of cholesterol in the body (Kucuk et al., 2017). Furthermore, the accumulation of cholesterol followed by free radical activity causes oxidative damage to several tissues. High cholesterol levels in the blood cause Very Low-Density Lipoprotein (VLDL) to form LDL, increasing LDL. LDL levels that continue to increase make HDL depressed and unable to get rid of excess cholesterol in the blood so that the HDL state decreases. This situation follows Andriani et al. (2020) statement that hypercholesterolemia results in impaired lipoprotein metabolism, including increased levels of LDL and decreased HDL levels. Maysghurt supplementation in hypercholesterolemic rats with four different doses showed a very significant difference (P < 0.05). There was an increase in HDL levels, and a decrease in LDL levels in hypercholesterolemic rats fed the maysghurt diet. The content of active compounds in corn is thought to affect these conditions. The results obtained are following several studies that the content of active compounds, including carotenoids and flavonoids, can help the body control cholesterol levels in the body (do Nascimento et al., 2020; Millar et al., 2017; Chávez-Santoscoy et al., 2013; Voutilainen et al., 2006; Arai et al., 2000; Dugas et al., 1998), in addition to increasing and facilitating blood circulation in

the body. The presence of active flavonoid compounds can increase the synthesis of bile acids (Millar et al., 2017; Chávez-Santoscoy et al., 2013).

The state of hypercholesterolemia causes the accumulation of excess fat, which will increase cholesterol levels. Excess fat and cholesterol cause chylomicrons to be converted into LDL by the lipoprotein lipase enzyme. Maysghurt supplementation made from corn can increase bile acid secretion, which will increase fat metabolism. As a result, excess fat will be excreted through the large intestine in the form of faeces. The removed fat will reduce cholesterol levels in the blood, the formation of LDL will also not be excessive. The mechanism for reducing LDL levels is influenced by lactic acid bacteria, namely Lactobacillus fermentum, which is found in maysghurt. Maysghurt fed to experimental rats contained approximately 7.9 log CFU/mL of lactic acid bacteria.

There are still limitations in this study, one of which is the absence of an analysis of the bioactive substances beforehand on maysghurt. This results in an unknown number of components of bioactive substances that may play a role in reducing LDL cholesterol levels in this study. However, as a fermented product, maysghurt has fulfilled several requirements that must be possessed by a product to be considered a functional food, namely: (1) Must be a food product (not in the form of capsules, tablets, or powder) derived from natural ingredients (ingredients), (2) Can and should be consumed as part of the daily diet or menu, (3) Has a specific function when digested, and can play a role in specific body processes, such as: strengthening the body's defence mechanism, preventing certain diseases, helping to restore the body's condition after certain illnesses, maintaining physical and mental condition, and slowing down the ageing process (Iwatani & Yamamoto, 2019; Pastrana et al., 2017; Kanekanian, 2014). From this concept, functional food is not the same as food supplements or drugs. Functional food can be consumed without a specific dose, can be enjoyed as food in general, and is delicious and nutritious (Alongi & Anese, 2021; Birch & Bonwick, 2019).

Conclusion :

Maysghurt, as a fermented product by *Lactobacillus fermentum*, has the prospect to be developed as a functional food because it meets sensory, nutritional and physiological requirements. Giving maysghurt to hypercholesterolemic rats for 21 days reduced total, and LDL cholesterol levels and increased HDL levels, and this intervention was also able to reduce the LDL/HDL ratio significantly. Further studies are needed regarding the effect of maysghurt on HDL levels, considering that this lipoprotein is a protective factor against atherosclerosis, the cause of cardiovascular disease. In addition, it is necessary to pioneer the trial of giving maysghurt to humans because it can be an alternative diet for hypercholesterolemic patients, which is relatively cheap and safe.

Acknowledgements

This paper is abstracted from a research project funded by an internal competitive grant at the State Islamic University of Alauddin. We want to thank the Head of the Biology Laboratory, Faculty of Science and Technology, UIN Alauddin Makassar and the entire research support team dedicated to this work.

Conflict of interest :

The author(s) declared no potential conflicts of interest concerning this article's research, authorship, and publication.

Reference :

- Adebola, O. O., Corcoran, O., & Morgan, W. A. (2020). Prebiotics may alter bile salt hydrolase activity: Possible implications for cholesterol metabolism. *PharmaNutrition*, *12*, 100182. https://doi.org/10.1016/j.phanu.2020.100182.
- Aguilar-Ballester, M., Herrero-Cervera, A., Vinué, Á., Martínez-Hervás, S., & González-Navarro, H. (2020). Impact of Cholesterol Metabolism in Immune Cell Function and Atherosclerosis. *Nutrients*, *12*(7), 2021. https://doi.org/10.3390/nu12072021.
- 3. Aloğlu, H., & Öner, Z. (2006). Assimilation of cholesterol in broth, cream, and butter by probiotic bacteria. *European Journal of Lipid Science and Technology*, *108*(9), 709–713. https://doi.org/10.1002/ejlt.200600137.
- 4. Alongi, M., & Anese, M. (2021). Re-thinking functional food development through a holistic approach. *Journal of Functional Foods, 81,* 104466. https://doi.org/10.1016/j.jff.2021.104466.
- 5. Andriani, R. D., Rahayu, P. P., & Apriliyani, M. W. (2020). The Effect of Probiotic in Milk Fermentation Towards Decreasing Cholesterol Levels: in Vivo Studies. *Jurnal Ilmu Dan Teknologi Hasil Ternak*, *15*(1), 13–20. https://doi.org/10.21776/ub.jitek.2020.015.01.2.
- 6. Annunziata, A., & Vecchio, R. (2013). Consumer perception of functional foods: A conjoint analysis with probiotics. *Food Quality and Preference, 28*(1), 348–355. https://doi.org/10.1016/j.foodqual.2012.10.009.
- 7. AOAC. (2005). Official Method of Analysis. 18th Edition. *AOAC Press, Maryland, USA*, 1(Volume 1).
- Arai, Y., Watanabe, S., Kimira, M., Shimoi, K., Mochizuki, R., & Kinae, N. (2000). Dietary Intakes of Flavonols, Flavones and Isoflavones by Japanese Women and the Inverse Correlation between Quercetin Intake and Plasma LDL Cholesterol Concentration. *The Journal of Nutrition*, *130*(9), 2243–2250. https://doi.org/10.1093/jn/130.9.2243.
- Astawan, M., Wresdiyati, T., Suliantari, S., Arief, I. I., & Septiawan, R. (2012). Production of Synbiotic Yogurt-Like Using Indigenous Lactic Acid Bacteria as Functional Food. *Media Peternakan*, 35(1), 9–14. https://doi.org/10.5398/medpet.2012.35.1.9.
- Baila-Rueda, L., Mateo-Gallego, R., Jarauta, E., de Castro-Orós, I., Bea, A. M., Cenarro, A., & Civeira, F. (2014). Bile acid synthesis precursors in familial combined hyperlipidemia: The oxysterols 24S-hydroxycholesterol and 27-hydroxycholesterol. *Biochemical and Biophysical Research Communications*, 446(3), 731–735. https://doi.org/10.1016/j.bbrc.2013.12.131.
- 11. Bernal Castro, C. A., Díaz-Moreno, C., & Gutiérrez-Cortés., C. (2017). Probióticos y prebióticos en matrices de origen vegetal: avances en el desarrollo de bebidas de frutas. *Revista Chilena de Nutrición*, 44(4), 383–392. https://doi.org/10.4067/S0717-75182017000400383.
- 12. Bhat, B., Habib, B., Bhagat, N., & Bajaj, B. K. (2019). Cholesterol-lowering and antioxidant potential of probiotic bacteria isolated from locally fermented milk product kalarei. *Indian Journal of Biochemistry and Biophysics*, *56*(5).
- 13. Birch, C. S., & Bonwick, G. A. (2019). Ensuring the future of functional foods. *International Journal of Food Science & Technology*, *54*(5), 1467–1485. https://doi.org/10.1111/ijfs.14060.
- 14. Brookfield, D. (2009). The Role Of Texture Analysis In Food Manufacturing. *Food Online*.

- Caponio, G. R., Wang, D. Q. H., Di Ciaula, A., De Angelis, M., & Portincasa, P. (2020). Regulation of Cholesterol Metabolism by Bioactive Components of Soy Proteins: Novel Translational Evidence. *International Journal of Molecular Sciences*, 22(1), 227. https://doi.org/10.3390/ijms22010227.
- Carson, J. A. S., Lichtenstein, A. H., Anderson, C. A. M., Appel, L. J., Kris-Etherton, P. M., Meyer, K. A., Petersen, K., Polonsky, T., & Van Horn, L. (2020). Dietary Cholesterol and Cardiovascular Risk: A Science Advisory From the American Heart Association. *Circulation*, 141(3). https://doi.org/10.1161/CIR.0000000000000743.
- Chávez-Santoscoy, R. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2013). Effect of Flavonoids and Saponins Extracted from Black Bean (Phaseolus vulgaris L.) Seed Coats as Cholesterol Micelle Disruptors. *Plant Foods for Human Nutrition*, 68(4), 416–423. https://doi.org/10.1007/s11130-013-0384-7.
- Cheng, P., Pan, J., Xia, J., Deng, F., Huang, W., Bai, S., Zhu, X., Shao, W., Wang, H., & Xie, P. (2018). Dietary cholesterol intake and stroke risk: a meta-analysis. *Oncotarget*, 9(39), 25698–25707. https://doi.org/10.18632/oncotarget.23933.
- 19. Chiang, J. Y. L. (1998). Regulation of bile acid synthesis. *Frontiers in Bioscience*, *3*(4), A273. https://doi.org/10.2741/A273.
- Choi, S.-B., Lew, L.-C., Yeo, S.-K., Nair Parvathy, S., & Liong, M.-T. (2015). Probiotics and the BSH-related cholesterol-lowering mechanism: a Jekyll and Hyde scenario. *Critical Reviews in Biotechnology*, 35(3), 392–401. https://doi.org/10.3109/07388551.2014.889077.
- Costa, M. P., Monteiro, M. L. G., Frasao, B. S., Silva, V. L. M., Rodrigues, B. L., Chiappini, C. C. J., & Conte-Junior, C. A. (2017). Consumer perception, health information, and instrumental parameters of cupuassu (Theobroma grandiflorum) goat milk yoghurts. *Journal of Dairy Science*, 100(1), 157–168. https://doi.org/10.3168/jds.2016-11315.
- 22. Deng, R. (2009). Food and food supplements with hypocholesterolemic effects. *Recent Patents on Food, Nutrition & Agriculture, 1*(1), 15–24. https://doi.org/10.2174/2212798410901010015.
- Di Taranto, M. D., de Falco, R., Guardamagna, O., Massini, G., Giacobbe, C., Auricchio, R., Malamisura, B., Proto, M., Palma, D., Greco, L., & Fortunato, G. (2019). Lipid profile and genetic status in a familial hypercholesterolemia pediatric population: exploring the LDL/HDL ratio. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 57(7), 1102–1110. https://doi.org/10.1515/cclm-2018-1037.
- 24. do Nascimento, T. C., Cazarin, C. B. B., Maróstica, M. R., Mercadante, A. Z., Jacob-Lopes, E., & Zepka, L. Q. (2020). Microalgae carotenoids intake: Influence on cholesterol levels, lipid peroxidation and antioxidant enzymes. *Food Research International*, *128*, 108770. https://doi.org/10.1016/j.foodres.2019.108770.
- Dugas, T. R., Morel, D. W., & Harrison, E. H. (1998). Impact of LDL carotenoid and α-tocopherol content on LDL oxidation by endothelial cells in culture. *Journal of Lipid Research*, *39*(5), 999–1007. https://doi.org/10.1016/S0022-2275(20)33867-0.
- 26. Félix-Redondo, F. J., Grau, M., & Fernández-Bergés, D. (2013). Cholesterol and cardiovascular disease in the elderly. Facts and gaps. In *Aging and Disease* (Vol. 4, Issue 3).
- Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clinical Chemistry*, 18(6), 499–502. https://doi.org/10.1093/clinchem/18.6.499.
- 28. Gérard, P. (2013). Metabolism of Cholesterol and Bile Acids by the Gut Microbiota. Pathogens,

3(1), 14–24. https://doi.org/10.3390/pathogens3010014.

- 29. Hansel, B., & Giral, P. (2015). Cholestérol alimentaire et morbi/mortalité cardiovasculaire. *OCL*, 22(2), D202. https://doi.org/10.1051/ocl/2015001.
- Horáčková, Š., Plocková, M., & Demnerová, K. (2018). Importance of microbial defence systems to bile salts and mechanisms of serum cholesterol reduction. *Biotechnology Advances*, 36(3), 682–690. https://doi.org/10.1016/j.biotechadv.2017.12.005.
- 31. Hu, F. B. (2005). Protein, body weight, and cardiovascular health. *The American Journal of Clinical Nutrition*, 82(1), 242S-247S. https://doi.org/10.1093/ajcn.82.1.242S.
- 32. Hu, Y., Xu, J., Chen, Q., Liu, M., Wang, S., Yu, H., Zhang, Y., & Wang, T. (2020). Regulation effects of total flavonoids in Morus alba L. on hepatic cholesterol disorders in orotic acid-induced NAFLD rats. *BMC Complementary Medicine and Therapies*, 20(1), 257. https://doi.org/10.1186/s12906-020-03052-w.
- Ishimwe, N., Daliri, E. B., Lee, B. H., Fang, F., & Du, G. (2015). The perspective on cholesterollowering mechanisms of probiotics. *Molecular Nutrition & Food Research*, 59(1), 94–105. https://doi.org/10.1002/mnfr.201400548.
- 34. Iwatani, S., & Yamamoto, N. (2019). Functional food products in Japan: A review. *Food Science and Human Wellness*, *8*(2), 96–101. https://doi.org/10.1016/j.fshw.2019.03.011.
- Janzantti, N. S., Machado, T. V., & Monteiro, M. (2011). Sensory acceptance of juice from food processing steps. *Journal of Sensory Studies*, 26(5), 322–330. https://doi.org/10.1111/j.1745-459X.2011.00347.x.
- Jesch, E. D., & Carr, T. P. (2017). Food ingredients that inhibit cholesterol absorption. In *Preventive Nutrition and Food Science* (Vol. 22, Issue 2). https://doi.org/10.3746/pnf.2017.22.2.67.
- 37. Joyce, S. A., MacSharry, J., Casey, P. G., Kinsella, M., Murphy, E. F., Shanahan, F., Hill, C., & Gahan, C. G. M. (2014). Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proceedings of the National Academy of Sciences*, 111(20), 7421–7426. https://doi.org/10.1073/pnas.1323599111.
- Kanekanian, A. (2014). Milk and Dairy Products as Functional Foods. In A. Kanekanian (Ed.), *Milk and Dairy Products as Functional Foods*. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118635056.
- 39. Karneta, R. (2019). Diversifikasi pengolahan jagung ketan merah (Zea mays ceratina) menjadi yogurt dengan fortifikasi susu skim dan sukrosa. *Seminar Nasional Lahan Suboptimal*.
- 40. Khare, A., & Gaur, S. (2020). Cholesterol-Lowering Effects of Lactobacillus Species. *Current Microbiology*, 77(4), 638–644. https://doi.org/10.1007/s00284-020-01903-w.
- Kim, S.-J., Park, S., Sin, H.-S., Jang, S.-H., Lee, S.-W., Kim, S.-Y., Kwon, B., Yu, K.-Y., Kim, S., & Yang, D. (2017). Hypocholesterolemic Effects of Probiotic Mixture on Diet-Induced Hypercholesterolemic Rats. *Nutrients*, *9*(3), 293. https://doi.org/10.3390/nu9030293.
- 42. Kingkaew, E., & Tanasupawat, S. (2019). Bile salt hydrolase activity of lactic acid bacteria: A potential for health. In *The Many Benefits of Lactic Acid Bacteria*.
- Kjeldsen, E. W., Nordestgaard, L. T., & Frikke-Schmidt, R. (2021). HDL Cholesterol and Non-Cardiovascular Disease: A Narrative Review. *International Journal of Molecular Sciences*, 22(9), 4547. https://doi.org/10.3390/ijms22094547.
- Kucuk, A., Uğur Uslu, A., Icli, A., Cure, E., Arslan, S., Turkmen, K., Toker, A., & Kayrak, M. (2017). The LDL/HDL ratio and atherosclerosis in ankylosing spondylitis. *Zeitschrift Für Rheumatologie*, *76*(1), 58–63. https://doi.org/10.1007/s00393-016-0092-4.
- 45. Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., Chakraborty, C., Singh,

B., Marotta, F., Jain, S., & Yadav, H. (2012). Cholesterol-Lowering Probiotics as Potential Biotherapeutics for Metabolic Diseases. *Experimental Diabetes Research*, 2012, 1–14. https://doi.org/10.1155/2012/902917.

- Kumar, R., Grover, S., & Batish, V. K. (2012). Bile Salt Hydrolase (Bush) Activity Screening of Lactobacilli: In Vitro Selection of Indigenous Lactobacillus Strains with Potential Bile Salt Hydrolysing and Cholesterol-Lowering Ability. *Probiotics and Antimicrobial Proteins*, 4(3), 162–172. https://doi.org/10.1007/s12602-012-9101-3.
- 47. Kundu, S. K., Khan, M. A. H. N. A., & Das, S. K. (2021). Cow Brain Consumption Causes Hypercholesterolemia: An in Vivo Study. *Asian Journal of Dairy and Food Research, Of.* https://doi.org/10.18805/ajdfr.DR-216.
- Kunutsor, S. K., Zaccardi, F., Karppi, J., Kurl, S., & Laukkanen, J. A. (2017). Is High Serum LDL/HDL Cholesterol Ratio an Emerging Risk Factor for Sudden Cardiac Death? Findings from the KIHD Study. *Journal of Atherosclerosis and Thrombosis*, 24(6), 600–608. https://doi.org/10.5551/jat.37184.
- 49. Lafuente, A., & De las Heras, L. (2018). Lípids. Nutrición Biomedicina UB, Mar Grasa.
- Leidy, H. J., Clifton, P. M., Astrup, A., Wycherley, T. P., Westerterp-Plantenga, M. S., Luscombe-Marsh, N. D., Woods, S. C., & Mattes, R. D. (2015). The role of protein in weight loss and maintenance. *The American Journal of Clinical Nutrition*, 101(6), 1320S-1329S. https://doi.org/10.3945/ajcn.114.084038.
- Liong, M. T., & Shah, N. P. (2005). Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. *International Dairy Journal*, 15(4), 391–398. https://doi.org/10.1016/j.idairyj.2004.08.007.
- 52. Liu, H., Huang, L., & Pei, X. (2021). Effects of sorghum rice and black rice on genes associated with cholesterol metabolism in hypercholesterolemic mice liver and intestine. *Food Science & Nutrition*, *9*(1), 217–229. https://doi.org/10.1002/fsn3.1986.
- MacDonald, C.-J., Madika, A.-L., Bonnet, F., Fagherazzi, G., Lajous, M., & Boutron-Ruault, M.-C. (2020). Cholesterol and Egg Intakes, and Risk of Hypertension in a Large Prospective Cohort of French Women. *Nutrients*, *12*(5), 1350. https://doi.org/10.3390/nu12051350.
- Millar, C. L., Duclos, Q., & Blesso, C. N. (2017). Effects of Dietary Flavonoids on Reverse Cholesterol Transport, HDL Metabolism, and HDL Function. *Advances in Nutrition: An International Review Journal*, 8(2), 226–239. https://doi.org/10.3945/an.116.014050
- 55. Mitsuoka, T. (2014). Development of Functional Foods. *Bioscience of Microbiota, Food and Health*, *33*(3), 117–128. https://doi.org/10.12938/bmfh.33.117.
- Moser, S. A., & Savage, D. C. (2001). Bile Salt Hydrolase Activity and Resistance to Toxicity of Conjugated Bile Salts Are Unrelated Properties in Lactobacilli. *Applied and Environmental Microbiology*, 67(8), 3476–3480. https://doi.org/10.1128/AEM.67.8.3476-3480.2001.
- Nuhwa, R., Tanasupawat, S., Taweechotipatr, M., Sitdhipol, J., & Savarajara, A. (2019). Bile salt hydrolase activity and cholesterol assimilation of lactic acid bacteria isolated from flowers. *Journal of Applied Pharmaceutical Science, 9*(6), 106–110. https://doi.org/10.7324/JAPS.2019.90615.
- Oh, S., Chai, C.-H., Kim, S.-H., Kim, Y.-J., Kim, H.-S., & Worobo, R. W. (2012). Comparison of Acid and Bile Tolerances, Cholesterol Assimilation, and CLA Production in Probiotic Lactobacillus acidophilus Strains. *Korean Journal for Food Science of Animal Resources*, 32(4), 409–413. https://doi.org/10.5851/kosfa.2012.32.4.409.
- 59. Pastrana, L., González, R., Estévez, N., Pereira, L., Rodríguez Amado, I., Fuciños, P., Fuciños, C., Rúa, M. L., Alonso, E., & Troncoso, R. (2017). Functional Foods. In *Current Developments in*

Biotechnology and Bioengineering (pp. 165–200). Elsevier. https://doi.org/10.1016/B978-0-444-63666-9.00007-8.

- Pavlović, N., Stankov, K., & Mikov, M. (2012). Probiotics—Interactions with Bile Acids and Impact on Cholesterol Metabolism. *Applied Biochemistry and Biotechnology*, *168*(7), 1880– 1895. https://doi.org/10.1007/s12010-012-9904-4.
- Pereira, D. I. A., & Gibson, G. R. (2002). Cholesterol Assimilation by Lactic Acid Bacteria and Bifidobacteria Isolated from the Human Gut. *Applied and Environmental Microbiology*, 68(9), 4689–4693. https://doi.org/10.1128/AEM.68.9.4689-4693.2002.
- 62. Piaggi, P. (2019). Metabolic Determinants of Weight Gain in Humans. *Obesity*, 27(5), 691–699. https://doi.org/10.1002/oby.22456.
- Pramono, Y. B., Dwiloka, N. B., Mulyani, S., Setiani, B. E., Rochmayani, M., & Bahtiar, D. E. (2020). Utilization of Lesser Yam (Dioscorea esculenta L.) Flour as Prebiotic in Yogurt to Total Lactic Acid Bacteria (LAB), Sugar Reduction, and Organoleptic Properties. *Digital Press Life Sciences*, 2, 00011. https://doi.org/10.29037/digitalpress.22325.
- Rahayu, P. P., & Andriani, R. D. (2018). Mutu Organoleptik dan Total Bakteri Asam Laktat Yogurt Sari Jagung dengan Penambahan Susu Skim dan Karagenan. *Jurnal Ilmu Dan Teknologi Hasil Ternak*, 13(1), 38–45. https://doi.org/10.21776/ub.jitek.2018.013.01.4.
- 65. Ramchandran, L., & Shah, N. P. (2011). Yoghurt Can Beneficially Affect Blood Contributors of Cardiovascular Health Status in Hypertensive Rats. *Journal of Food Science*, *76*(4), H131–H136. https://doi.org/10.1111/j.1750-3841.2011.02127.x.
- Saikia, D., Manhar, A. K., Deka, B., Roy, R., Gupta, K., Namsa, N. D., Chattopadhyay, P., Doley, R., & Mandal, M. (2018). Hypocholesterolemic activity of indigenous probiotic isolate Saccharomyces cerevisiae ARDMC1 in a rat model. *Journal of Food and Drug Analysis*, *26*(1), 154–162. https://doi.org/10.1016/j.jfda.2016.12.017.
- 67. Sarita. (2012). Kandungan kolesterol dalam berbagai bahan makanan hewani. *Indonesian Bulletin of Health Research, 27*(2). https://doi.org/10.22435/bpk.v27i2Jun.305.
- 68. Sarkar, S. (2008). Effect of probiotics on biotechnological characteristics of yoghurt. *British Food Journal*, *110*(7), 717–740. https://doi.org/10.1108/00070700810887185.
- 69. Sengupta, S., Koley, H., Dutta, S., & Bhowal, J. (2019). Hepatoprotective effects of synbiotic soy yoghurt on mice fed a high-cholesterol diet. *Nutrition*, 63–64, 36–44. https://doi.org/10.1016/j.nut.2019.01.009.
- 70. Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal*, 17(11), 1262–1277. https://doi.org/10.1016/j.idairyj.2007.01.014.
- 71. Shiby, V. K., & Mishra, H. N. (2013). Fermented Milk and Milk Products as Functional Foods— A Review. *Critical Reviews in Food Science and Nutrition*, *53*(5), 482–496. https://doi.org/10.1080/10408398.2010.547398.
- Singh, T. P., Malik, R. K., Katkamwar, S. G., & Kaur, G. (2015). Hypocholesterolemic effects of Lactobacillus reuteri LR6 in rats fed on high-cholesterol diet. *International Journal of Food Sciences and Nutrition*, 66(1), 71–75. https://doi.org/10.3109/09637486.2014.953450.
- 73. Soliman, G. (2018). Dietary Cholesterol and the Lack of Evidence in Cardiovascular Disease. *Nutrients*, *10*(6), 780. https://doi.org/10.3390/nu10060780.
- 74. Supavititpatana, P., Wirjantoro, T. I., & Raviyan, P. (2010). Characteristics and shelf-life of Corn Milk Yogurt. *Chiang Mai University Journal of Natural Sciences*.
- 75. Trikoomdun, W., & Leenanon, B. (2016). Production of corn milk yoghurt supplemented with probiotics. *International Food Research Journal*, *23*(4), 1733–1738.
- 76. Vedaraman, N., Srinivasakannan, C., Brunner, G., Ramabrahmam, B. V., & Rao, P. G. (2005).

Experimental and modelling studies on extraction of cholesterol from cow brain using supercritical carbon dioxide. *The Journal of Supercritical Fluids*, *34*(1), 27–34. https://doi.org/10.1016/j.supflu.2004.10.004.

- 77. Voutilainen, S., Nurmi, T., Mursu, J., & Rissanen, T. H. (2006). Carotenoids and cardiovascular health. *The American Journal of Clinical Nutrition*, *83*(6), 1265–1271. https://doi.org/10.1093/ajcn/83.6.1265.
- 78. Walker, J. (2013). Reducing cardiovascular disease risk: cholesterol and diet. *Nursing Standard*, *28*(2), 48–55. https://doi.org/10.7748/ns2013.09.28.2.48.e7747.
- 79. Welli, Y., Agnes, M., Yudi, P., & Yustinus, M. (2019). The effect of application of cavendish Jepara 30 banana pseudostem flour on the production of short-chain fatty acids and cholesterol in caecum digesta of hypercholesterolemic mice. *International Journal of Scientific and Technology Research*, *8*(12).
- Widodo, W., Fanani, T. H., Fahreza, M. I., & Sukarno, A. S. (2021). Cholesterol Assimilation of Two Probiotic Strains of Lactobacillus casei used as Dairy Starter Cultures. *Applied Food Biotechnology*, 8(2). https://doi.org/10.22037/afb.v8i2.30661.
- Xu, Z., McClure, S., & Appel, L. (2018). Dietary Cholesterol Intake and Sources among U.S Adults: Results from National Health and Nutrition Examination Surveys (NHANES), 2001– 2014. *Nutrients*, 10(6), 771. https://doi.org/10.3390/nu10060771.
- Yadav, H., Jain, S., & Sinha, P. R. (2007). Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose-fed rats. *Nutrition*, 23(1), 62– 68. https://doi.org/10.1016/j.nut.2006.09.002.