Jurnal Aisyah: Jurnal Ilmu Kesehatan

Volume 8, Issue 1, March 2023, p. 39–42
ISSN 2502-4825 (print), ISSN 2502-9495 (online)

Differences effect of tempeh milk and tempeh yogurt on oxidative stress in maximal exercise

Anggit Mustika Ningrum[1], Hardhono Susanto [2*)], Etika Ratna Noer [3], Muflihatul Muniroh[4], Diana Nur Afifah[5]

[1,3,5] Science of Nutrition Department, Faculty of Medicine, Diponegoro University
[2,4] Medical Department, Faculty of Medicine, Diponegoro University, Semarang

ARTICLE INFO

Article history:

Received 10 October 2022
Accepted 31 January 2023
Published 20 March 2023

Keyword:

tempeh milk
tempeh yoghurt
oxidative stress

A B S T R A C T

Maximum exercise can cause oxidative stress which is characterized by high levels of MDA and decreased SOD enzyme activity. Tempeh milk and tempeh yogurt are natural sources of antioxidants that can prevent damage by free radicals and excess ROS formation. This study aimed to analyze the effect of tempeh milk and tempeh yogurt on oxidative stress in rats with maximum exercise. This study is a true experimental with a pre-test post-test control group design on 28 rats given maximal physical activity and treatment for 10 days which were divided into four groups, the control group without maximal physical activity treatment, the control group with maximal activity treatment, the tempeh milk intervention group and the tempeh yogurt intervention group. The statistical analysis used was paired t-test and one-way ANOVA test. The intervention of tempeh milk (4.2 ml/weight of rats) and tempeh yogurt (4.4 ml/weight of rats) had a significant effect ($p<0.05$) on decreasing MDA levels, and also had a significant effect ($p<0.05$) on increasing SOD enzyme activity in rats with oxidative stress. In conclusion, tempeh milk and tempeh yoghurt can reduce MDA levels and increase SOD enzyme activity

Kata kunci:

susu tempe
yoghurt tempe
stress oksidatif

**) corresponding author*

Prof. Dr. dr. Hardhono Susanto, PAK

Medical Department, Faculty of Medicine, Diponegoro University, Semarang
Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50271

Email: hardhonosusanto@yahoo.com

DOI: 10.30604/jika.v8i1.1561

A B S T R A K

Latihan fisik maximal dapat memicu stres oksidatif dengan ditandai tingginya kadar MDA serta aktivitas enzim SOD yang menurun. Susu tempe dan yoghurt tempe menjadi salah satu pangan sumber antioksidan alami yang dapat dimanfaatkan untuk mencegah terjadinya kerusakan yang disebabkan oleh radikal bebas dan pembentukan ROS secara berlebih. Tujuan penelitian ini, untuk menganalisis pengaruh dari susu tempeh dan yoghurt tempeh terhadap stres oksidatif pada tikus dengan latihan fisik maksimal. Penelitian ini merupakan true experimental dengan pre-test post-test control group desain pada 28 ekor tikus yang diberikan aktivitas fisik maximal dan perlakuan selama 10 hari yang dibagi menjadi 4 kelompok, kelompok kontrol tanpa perlakuan aktivitas fisik maximal, kelompok kontrol dengan perlakuan aktivitas fisik maximal, kelompok intervensi susu tempe dan kelompok intervensi yoghurt susu tempe. Analisis statistik yang digunakan adalah uji paired t-test dan uji one-way ANOVA. Intervensi susu tempeh (4,2ml/BB tikus) dan yoghurt tempeh (4,4ml/BB tikus) berpengaruh secara signifikan ($p<0.05$) terhadap penurunan kadar MDA, dan juga berpengaruh signifikan *($p<0.05$)* pada peningkatan aktivitas enzim SOD tikus dengan stress oksidatif. Sehingga dapat disimpulkan bahwa susu tempe dan yoghurt tempe dapat menurunkan kadar MDA serta meningkatkan aktivitas enzim SOD

INTRODUCTION

Physical exercise is properly can provide benefits to the body and improve performance, but nowadays it is reported that professional athletes do more physical exercise than is recommended. Physical exercise given to experimental animals with weights can increase Reactive Oxygen Species (Suarsana,2013). Exposure to ROS on lipid cell membranes results in fatty acid peroxidation which is characterized by high lipid peroxidation end products, namely malondialdehyde (Hudson et al., 2008). Increased free radical can affect the decrease in the SOD enzyme activity which is the primary antioxidant catalyzing superoxide radicals into intracellular peroxides, to neutralize the effects of free radicals, higher amounts of exogenous antioxidants are needed (Amitava,2014).

Exogenous antioxidant intake in athletes has an important role in reducing oxidative stress, one of which is isoflavones which can help the performance of intracellular antioxidant enzymes to prevent free radical cells (Kerksick et al, 2018). Intake of antioxidants in athletes can be given in the form of sports drinks to be able to complement the daily nutritional. The results of the research that sport drinks can improve oxidative stress are indicated by the decreased value of MDA levels (Santos et al, 2016).

Tempeh yoghurt can be used as an alternative to sports drinks other than tempeh milk. The nutritional content of yoghurt depends on the ingredients used, tempeh yoghurt has a protein content of 14.30g/100g (Feng Tao, 2020). Tempeh milk as raw material for tempeh yoghurt is an alternative source of probiotics and prebiotics containing bioactive. Prebiotic content in tempeh can be used as a yoghurt substrate that can increase probiotics (Bintari et al, 2019). Probiotics have an important role in antioxidant capacity, oxidative damage repair system by reducing free radicals in the body and modulating intestinal microbiota. Some probiotics can increase the activity of antioxidant enzymes and reduce oxidative stress that can damage cells.

This prompted researcher's interest in determining to compare the effects of the two types of drinks in reducing oxidative stress on cell membranes due to maximal physical exercise. There are no studies that directly compare the effect of intake of tempeh milk and tempeh yoghurt on MDA levels and SOD enzyme activity.

METHODS

Animals

The animals used in this study were male Sprague Dawley rats aged 2-3 months with its weight around 150-200 grams. Experimental animals totalling 28 rats were obtained from the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. This research had been approved by the Health Research Ethics Commission, Faculty of Medicine, Diponegoro University, No. 56/EC/H/FKUNDIP/VII/2022

Research design

This research was conducted by a true experimental approach with a randomized pre and post-test control group design. A total of 28 rats were randomly divided into four groups. The negative group (K-) : standard feed, the positive group (K+) : with excertise without treatment, treatment group 1 (P1) : tempeh milk and treatment group 2 (P2) : tempeh yoghurt

Materials and methods

The design in this study was experimental with a completely randomized design. There were two treatments. The manufacture of synbiotic tempeh yoghurt is carried out at the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.

Tempeh milk

The start from tempeh cut into cubes with a size of ± 1.5 x 1.5 x 1.5 cm, proses of making tempeh and then steam blanching at a temperature of 80º-100ºC for 10 minutes. After that, using a blender tempeh is then mashed and then added water at a ratio of 1:2 tempeh and water to obtain tempeh milk. Furthermore, a nutritional analysis of tempeh milk was carried out which included a proximate test. The raw material for tempeh milk is tempeh which comes from a home industry from Sleman, Yogyakarta.

Tempeh yoghurt

The process of making tempeh yoghurt starts from tempeh milk, tempeh milk is heated at a temperature of 70-80℃ then cooled to 40℃ then inoculated aseptically with a commercial yoghurt starter with *steptococus termopilus* and *Lactobacilus bulgaricus* as much as 5% by volume of tempeh milk. Furthermore, it was incubated at 40℃ for 12 hours. Furthermore, a nutritional analysis of tempeh yoghurt was carried out which included a proximate test.

Maximal excercises intervention

Rats in this study were given swimming activities with an additional load of 6% of their body weight which was tied to the tail. Rats will swim in a plastic container (container) filled with water with a minimum height of 50 cm with monitored water temperature ranging from 33-36°C, with swimming duration set for the day of data collection is 10 minutes (Quinla,2012). Swimming sports are given in the positive group (K+), treatment group 1 (P1) and (P2) for 10 days.

Rats will be considered swimming if the rats swim with a regular rhythm, not tiptoeing *(bobbing)* or rough swimming rhythms *(climbing).*

Interventions of tempeh milk and tempeh yoghurt

Rats will be weighed in the morning before being given the intervention to adjust the dose to be given. The intervention will be given in liquid form 10 minutes before the rats are given the swimming exercise treatment. The intervention was given to rats in the form of a probe. The intervention dose given was following the results of previous studies with the best dose of effectiveness, namely the dose of tempeh 3g/BW rats. Rats after high activity conditioning were randomized and grouped into 4 groups, 1 positive control group, 1 negative control group and 2 groups treatment by giving tempeh milk and tempeh yoghurt with gastric sonde method and given once a day in the morning after maximal physical exercise. The dose of tempeh milk was given 4.2 ml/weight of rats and the dose of tempeh yoghurt was 4.4 ml/weight of rats, in each intervention dose contained 3g of tempeh. The intervention was carried out for

10 days, then the rats were drawn blood for examination of MDA levels and SOD enzyme activity (Ariani,2018).

Statistical Analysis

Statistical analysis was used SPSS 24 program. Paired t-test was used to determine the effect on each group before and after treatment. One-way ANOVA test was used to determine the differences in each group, followed by the Bonferroni post-hoc test. The statistical test was carried out at the 95% of confidence level (p<0.05).

RESULTS AND DISCUSSION

Nutrient content of tempeh milk and tempeh yoghurt

The tempeh milk used in this study has an energy content of 10.12 kcal, 0.37 gram protein, 0.91 gram fat, 0.10 gram carbohydrate and 0.14 gram fiber. Tempeh milk yoghurt contains 12.43 kcal of energy, 1.11 grams of protein, 0.82 grams of fat, 0.50 grams of carbohydrates and 0.38 grams of fiber. When compared, the nutritional content of tempeh milk yoghurt is higher than tempeh milk because of the addition of lactic acid bacteria starter. This is due to the increase in total solids so that the content of other nutrients increases. Yoghurt starter in the form of lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) grown in tempeh milk will cause the formation of several compounds. The addition of bacteria in the manufacture of this yoghurt triggers the fermentation process of tempeh milk.

Malondiadehide (MDA) and Superoxide Dismutase (SOD)

Examination of MDA levels was carried out after acclimatization on day 3 to show that MDA levels were normal when the rats had not been given maximum physical activity and before being given treatment. The changes in the mean levels of MDA before and after the intervention can be seen in Table 1.

Tabel 1 Average *Malondiadehide (MDA)* values before and after intervention

MDA (nmol/ml)	K- Average ± SD	K+ Average ± SD	P1 Average ± SD	P2 Average ± SD	p′
Before intervention	0.94 ± 0.15	1.11 ± 0.19	1.11 ± 0.22	1.08 ± 0.19	
After intervention	1.19 ± 0.15	10.42 ± 0.45	2.87 ± 0.47	2.10 ± 0.20	
Delta	-0.69 ± 0.11	0.97 ± 0.03	0.24 ± 0.09	-0.00 ± 0.12	**0.000**
Persentation (%)	28.62 ± 9.17	8.59 ± 182.38	1.64 ± 44.63	99.11 ± 36.79	
p	**0.000**	**0.000**	**0.000**	**0.000**	

Note: a paired t-test (p<0.05)

Based on the ANOVA test there was a significant difference between the control and treatment groups where (p'=0.000), this means that all groups at the beginning of the study before being given maximum physical activity and treatment had different and significant mean MDA levels. when compared with K+, namely the group that was only given high physical activity without any intervention, there was a significant difference in changes in the value of MDA levels in groups P1 (p=0.000) and P2 (p=0.001). High MDA levels indicate increased lipid peroxidation processes that occur, and high lipid peroxidation processes indicate high levels of free radicals in the body. MDA levels were higher in groups P1 and P2, this means that after maximal physical activity the rats were already in a state of oxidative stress. The high maximum physical activity carried out every day for 10 days results in high free radicals in the body where the number of endogenous antioxidants is strongly influenced by free radicals formed in the body during physical activity which will result in oxidative stress.

Interventions in the form of tempeh milk and tempeh milk yoghurt have been shown to reduce MDA levels. Bintari's research on 2019 shows that giving tempeh to experimental animals can optimally neutralize excessive free radicals in the body. This shows that tempeh milk yoghurt can prevent the formation of excess free radicals so that oxidative stress will not occur. Inhibited oxidative stress will prevent lipid peroxidation from forming which is indicated by a decrease in the value of MDA levels. Tempeh milk yoghurt also contains antioxidants that can prevent excess free radicals in the body so that oxidative stress can be avoided (Moore,2018). Inhibited oxidative stress results in lipid peroxidation. The P2 group with the tempeh milk yoghurt intervention showed a decrease in MDA levels compared to the P1 group with the tempeh milk intervention because the tempeh milk yoghurt contains bioactive (lactic acid bacteria) that work as antioxidants through the process of inhibiting lipid peroxidation with free radical scavengers (Pereira et al,2013)

Examination of the level of SOD enzyme activity was carried out 2 times, namely before and after the intervention of tempeh milk and tempeh milk yoghurt was given. Examination of SOD enzyme activity levels was carried out after acclimatization on day 3 as a reference to see the levels of SOD enzyme activity when rats had not been given maximum physical activity and before being given treatment. The first line of antioxidant enzymes is SOD enzyme activity, this enzyme is indispensable in all defenses from damage to biomolecules caused by free radicals.

Table 2. Average *Superoxide Dismutase (SOD)* values before and after intervention

SOD U/ml	K- Average ± SD	K+ Average ± SD	P1 Average ± SD	P2 Average ± SD	p′
Before intervention	84.55 ± 3.26	89.46 ± 5.17	90.86 ± 5.59	88.99 ± 6.67	
After intervention	81.33 ± 3.29	34.66 ± 2.39	58.64 ± 3.89	67.44 ± 4.58	
Delta	-3.22 ± 0.14	-54.79 ± 5.25	-32.23 ± 1.90	-21.54 ± 8.22	**0.000**
Persentation (%)	-3.81 ± 0.24	-61.17 ± 3.24	-35.48 ± 0.79	-23.84 ± 7.57	
P	0.000	0.000	0.000	0.000	

Note: a paired t-test (p<0.05)

Based on the ANOVA test result, there was a significant difference between the control and treatment groups where (p'=0.000), this means that all groups at the beginning of the study before being given maximal physical activity and treatment had different and significant mean levels of SOD enzyme activity. Provision of interventions in the form of tempeh milk and tempeh yoghurt has been shown to increase levels of SOD enzyme activity (Harun, 2017). Harun & Jauhari (2014) research showed that giving tempeh could increase the level of SOD enzyme activity. Giving tempeh as an antioxidant that has bioactive substances in the form of flavonoids which are considered free radical scavengers can increase the activity of the SOD enzyme (Retnaningsih, 2013). Flavonoids are reducing compounds that are able to inhibit various oxidation reactions, reduce the amount of ROS and increase the SOD enzyme activity. Flavonoids can help superoxide dismutase work in the body by counteracting free radicals formed due to oxidative stress (Asih et al., 2013). The results of this study are in line with Harun's 2017 research that flavonoid-rich tempeh at a dose of 3 g/head/day can inhibit formation of lipid peroxidation and maintain high SOD levels. Utami (2017) research states that consumption of tempeh can increase the activity of the SOD enzyme in the blood by 56.9%. Flavonoids contained in tempeh milk and tempeh milk yoghurt can increase the activity of the SOD enzyme.

CONCLUSIONS AND SUGGESTIONS

The administration of tempeh milk and tempeh yoghurt was significantly reduced the MDA and increase value in Sprague Dawley rats with maximal excercise. Suggestions for further research is necessary to conduct further examination of the totally antioxidant and isoflavon.

ETHICAL CONSIDERATIONS

Funding Statement

This research was funded by the UP3 FK UNDIP 2022 in a research thesis scholarship program

Conflict of Interest Statement

The author(s) declared no conflict of interest in this research

REFERENCES

Al-baarri, A. N., Legowo, A. M., Pramono, Y. B., Fazriyati, R. S., Pangestu, R. F., Azhar, H. N., Sarya, R. H., & Hapsari, M. C. (2016). Teknik Pembuatan Fruity Powder Yoghurt (Issue June).

Ariani, D., & Angwar, M. (2018). Produk Pangan Berbasis Tempeh dan Aplikasinya. Lembaga Ilmu Pengetahuan Indonesia (LIPI).

Amitava D, Kimberly K. (2014). Antioxidant in food, Vitamins and supplement preventions and treadment of disease. Elsivier Inc

Astuti, S., Muchtadi, D., Astawan, M., Purwantara, B., & Wresdiyati, T. (2009) Pengaruh Pemberian Tepung Kedelai Kaya Isoflavon Terhadap Kadar Malonaldehid (MDA), Aktivitas Superoksida Dismutase (SOD) Testis dan Profil Cu, Zn-SOD Tubuli Seminiferi Testis Tikus Jantan. Jurnal Teknologi dan Industri Pangan.; 20(2): 129.

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

Harun, I., Susanto, H., & Rosidi, A. (2017). Pemberian Tempeh Menurunkan Kadar Malondialdehyde (MDA) Dan Meningkatkan Aktivitas Enzim Superoxide Dismutase (SOD) Pada Tikus Dengan Aktivitas Fisik Tinggi. 12(11), 211– 216.

Hudson, M.B.; Hosick, P.A.; Mccaulley, G.O.; Schrieber, L.; Wrieden, J.; Mcanulty, et al. (2008). The Effect Of Resistance Exercise On Humoral Markers Of Oxidative Stress. Med. Sci. Sports Exerc. 40: 542–548. https://doi/org/10.1249/MSS.0b013e31815daf89

Jauhari M. (2014). Development of Tempeh Based Sports Beverages for Muscles Damage Recovery. Bogor: Jurnal Institut Pertanian Bogor.

Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, JagerR, et al. (2018). exercise & sports nutrition review update: Research & recommendations. J Int Soc Sports Nutr.15(1):1–57. https://doi.org/10.1186/s12970-018-0242-y.

Moore, J. B., Horti, A., & Fielding, B. A. (2018). Evaluation of the nutrient content of yoghurts: A comprehensive survey of yoghurt products in the major UK supermarkets. BMJ Open, 8(8), 1–11. https://doi.org/10.1136/bmjopen-2017-021387

Pereira, E., Barros, L., Ferreira, I. (2013). Relevance of the mention of antioxidant properties in yoghurt labels: In vitro evaluation and chromatographic analysis. Antioxidants. Journal Antioxidant. 2: 62-76; https://doi.org/:10.3390/antiox2020062

Retnaningsih, Christiana et al. (2013) Increased Superoxide Dismutase Antioxidant Activity in Hyperglycemia Rat with Velvet Bean (Mucuna pruriens L.) Tempeh Diet. Agritech Journal. Vol. 33, No. 2.

Suarsana In, Wresdiyati T, Suprayogi A.(2013). Response Of Oxidative Stress And Isoflavone Treatment On Superoxide Dismutase Enzyme Activities And Lipid Peroxidation In Rat′s Liver. Jitv. 18(2): 146-152

Utari DM. (2011). Efek intervensi tempeh terhadap profil lipid, superoksida dismutase, LDL teroksidasi dan malondialdehyde pada wanita menopause.[Thesis]. Bogor: Institut Pertanian Bogor.

Utami, Kiswanti. (2017). Potensi Pemberian Probiotik Lactobacillus Casei Strain Shirota Terhadap Kadar Kolesterol Total, Kadar Mda, Aktivitas Sod Dan Histopatologi Jaringan Aorta Pada Tikus Dengan Diet Tinggi Kolesterol. Universitas Brawijaya.

Widyastuti RA. (2019). Pemberian pengaruh sari kedelai dan susu coklat terhadap kadar laktat, kadar creatine kinase, kapasitas aerobik, dan daya anaerobik: studi pada atlet cabang olahraga sepakbola di pusat pelatihan dan Latihan olahraga pelajar jawa tengah. Semarang. Universitas Diponegoro.

Quinlan CL, et al. (2012). Mitochondrial complex II Can generate Raective Oxygen Species at high rates in both the forward and reverse reaction. J boil Chem. 287-299 //https://doi.org/: 10.1074/jbc.M112.374629