

Identification of LDH serum levels in male Wistar rats that were given a modified tempeh-based sports drink

MARDIANA¹, YANESTI NURAVIANDA LESTARI², EKO FARIDA³

^{1,2,3}Bachelor Nutrition Study Program, Sport Sciences Faculty, Semarang State University, INDONESIA

Published online: June 30, 2021

(Accepted for publication June 15, 2021)

DOI:10.7752/jpes.2021.04230

Abstract:

Protein is one of the most widely used supplements by athletes. Protein supplementation is used for both the recovery process and to improve exercise performance. One type of protein that can speed up the recovery process is branched-chain amino acids (BCAAs). BCAAs are a component that can have an impact on reducing the risk of muscle injury. BCAAs, along with citrulline and bromelain, can also reduce oxidative stress, which can cause muscle injury. This study aimed to analyze the effect of a tempeh-based sports drink on lactate dehydrogenase (LDH) levels of male Wistar rats. This in vivo study on 30 male Wistar rats aged 8–9 weeks weighing approximately 250 g used a posttest only control group design consisting of two control groups (K (–) and K (+)) and three treatment groups (P (1), P (2), and P (3)). The control group was given tempeh flour, and the other groups were given tempeh flour with watermelon and/or pineapple juice. All groups were subjected to exercise in the form of intense acute swimming for 30–40 minutes. The results showed that the plasma LDH levels 2 hours after treatment were significantly higher compared with the plasma LDH levels 24 hours after treatment ($p = 0.0001$). The difference in plasma LDH levels 2 hours and 24 hours after treatment showed a significant difference between the control group and the treatment group ($p = 0.006$). It can be concluded that the treatment group P (3) showed the most significant reduction in plasma LDH levels compared with the control group and other treatment groups.

Key Words: lactate dehydrogenase, muscle injury, branched-chain amino acid, citrulline, bromelain

Introduction

Muscle injury is an inevitable occurrence in sports activities. Muscle injury can occur because of sudden and/or heavy movements. Muscle injury can be induced by physical exercise that exceeds the body's capacity, which is termed exercise-induced muscle damage (EIMD) (Owens et al., 2019; Tipton, 2015; Mueller-Wohlfahrt et al., 2013). There are two causes of muscle injury: direct trauma, such as muscle lacerations, and indirect trauma, such as strains. More than 90% of sports injuries are caused by strains, whereas muscle lacerations are rare. Under strain conditions, the myofibrils are exposed to excessive internal tensile forces. The severity of strain-related injuries, such as delayed-onset muscle soreness (DOMS), varies. After minor trauma such as strains, skeletal muscles can regenerate completely and spontaneously, but in severe trauma, the muscle healing process can occur imperfectly, resulting in the formation of fibrotic tissue, which interferes with muscle function (Laumonier & Menetrey, 2016). Symptoms that indicate injury or muscle inflammation can include the swelling of muscle fibers and increased activity of the enzymes lactate dehydrogenase (LDH) and creatine kinase (CK), and even a decrease muscle strength and range of motion (ROM) of the knee joints (Tartibian et al., 2009; Meamarbashi, 2017).

To overcome this problem, many athletes use supplements and sports drinks, which can contain protein, herbs, and other bioactive components, one of which is *Nigella sativa*. However, based on study results, the administration of *Nigella sativa* at doses as high as 1500 and 3000 mg does not provide significantly faster recovery after EIMD (Riyanto et al., 2016; Raimi et al., 2020). Several other studies have shown that protein supplementation (whey, casein, soybeans, and eggs) and amino acids can increase protein synthesis, inhibit proteolysis, and reduce the risk of muscle inflammation and oxidative stress (Hulmi et al., 2010; Tang, 2009; Nicastro et al., 2012). Chronic branched-chain amino acid (BCAA) supplementation (4 weeks) reduced muscle damage, enhanced synthesis of muscle protein, and improved recovery from EIMD in 20 well-trained road cyclists (Shenoy et al., 2017). One potential source of BCAAs is tempeh. A serving of 100 grams of tempeh provides 96.97 mg of the amino acid lysine, 144.41 mg of isoleucine, and 89.98 mg of valine, all of which are BCAAs. The high BCAA content in tempeh makes it a promising supplement for the recovery of muscle damage after strength training (Rahmi et al., 2018; Jauhari et al., 2014).

Along with BCAAs, the amino acid citrulline and the protease enzyme can be used to aid in the recovery process of muscle injuries. Citrulline is a non-essential amino acid that improves athletic performance, reduces the risk of muscle soreness, and delays post-exercise muscle fatigue. When the body is exercising, ammonia levels increase, activate phosphofructokinase, and facilitate lactic acid production. Excessive lactic

acid interferes with muscle contraction and causes muscle fatigue. Citrulline can detoxify ammonia so that lactic acid production can be controlled and recycled into energy (gluconeogenesis) through the Cori cycle. Based on the results of a systematic review and meta-analysis that examined the effect of post-exercise citrulline supplementation on rating of perceived exertion (RPE), muscle soreness, and blood lactate levels, supplementation with 8 g of citrulline malate can reduce RPE and muscle soreness at 24 and 48 hours post-exercise without affecting blood lactic acid levels (Proia et al., 2016; Rhim et al., 2020). Another study indicated that giving watermelon juice rich in citrulline significantly reduced fatigue and muscle soreness after weight training (Sirait et al., 2016).

Not only have BCAAs and citrulline been shown to reduce the risk of muscle soreness and delay post-exercise muscle fatigue, but the enzyme bromelain can also delay fatigue and reduce the risk of muscle soreness. Bromelain is a sulfhydryl proteolytic enzyme contained in the stems, fruit, and cores of pineapple. Bromelain has been widely developed as an anti-inflammatory, immunomodulatory, anti-fibrinolytic, and anti-edema agent (Margaretta et al., 2015; Mameli et al., 2020). As an anti-inflammatory agent, bromelain can modulate the adhesion of macrophages, T cells, and NK cells and induce cytokine secretion (IL-1 β , IL-6, and TNF-) in vitro (Schulz et al., 2018; Chandanwale et al., 2017). A two-stage clinical trial administered the systemic enzyme therapy Wobenzym Plus (containing 67.5–76.5 mg bromelain, 32–48 mg trypsin, and 100 mg rutoside 3 H₂O; Mucos Pharma CmbH & Co. KG, Berlin, Germany) 72 hours before and 72 hours after exercise. This protocol reduced pressure-induced pain (PIP) and increased anti-inflammatory biomarkers in all participants (Marvin et al., 2017).

The purpose of proper nutrition during the recovery process after muscle injury is to accelerate the healing process and reduce the negative impact of decreased physical activity. Therefore, we are interested in conducting research using sports drinks made from tempeh and food ingredient sources of citrulline and bromelain, which we produced through trial-and-error development of material formulations. The purpose of this study was to determine the effect of a tempeh-based sports drink on LDH levels of male Wistar rats.

Material & Methods

This research was conducted using a true experimental method, which was carried out in two stages of research. The first stage of research used a completely randomized design with one factor: the addition of four types of tempeh/fruit juice sports drinks. The sports drinks were SPT1 (tempeh sports drink without fruit juice), SPT2 (tempeh sports drink with watermelon juice), SPT3 (tempeh sports drink with pineapple juice), and SPT4 (tempeh sports drink with watermelon and pineapple juice). The second stage of the study used a post-test-only control group design consisting of two control groups and three treatment groups. Research using experimental animals was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Semarang State University.

Sports Drink Development

A tempeh-based sports drink was developed, along with modified sports drinks with the addition of watermelon juice and/or pineapple juice.

Table 1. Composition of the tempeh-based sports drink.

| Ingredients | Treatments (n = 12) | | | |
|-----------------------|---------------------|------|------|------|
| | SPT1 | SPT2 | SPT3 | SPT4 |
| Tempeh flour (g) | 51 | 51 | 51 | 51 |
| Maltodextrin (g) | 43.5 | 43.5 | 43.5 | 43.5 |
| Water (mL) | 600 | - | - | - |
| Watermelon Juice (mL) | - | 600 | - | 300 |
| Pineapple Juice (mL) | - | - | 600 | 300 |

Treatments (n = 12): SPT1, tempeh sports drink without fruit juice; SPT2, tempeh sports drink with watermelon juice; SPT3, tempeh sports drink with pineapple juice; and SPT4, tempeh sports drink with watermelon and pineapple juice.

The tempeh-based sports drink was developed by mixing tempeh flour and maltodextrin and then adding 600 mL of liquid (water and/or watermelon juice and/or pineapple juice). The solution was then stirred until homogeneous and heated over low heat for approximately 10 minutes, then aerated and transferred to a packaging bottle. The complete composition of the tempeh sports drinks is presented in Table 1.

Use of Experimental Animals

This study used a sample of experimental animals, namely, white rats of the Wistar strain (*Rattus norvegicus*) that met the following criteria: 8–9 weeks of age, male sex, white hair, active appearance, a body weight of approximately 250 g, and no anatomical abnormalities. The mice that were unable to complete the exercise protocol were not included in the study sample. The research sample was selected using the simple random sampling method, and each group consisted of 5 rats so that the total number of rats in the study sample was 30. Before treatment, the rats were first adapted for 7 days and placed in a clean cage with daily access to food and drinking water ad libitum.

After the adaptation period, the rats were randomly grouped into five groups consisting of two control groups (K (+) = placebo and K (-) = given tempeh-only sports drink) and 3 treatment groups (P (1) = given

tempeh sports drink with watermelon juice, P (2) = given tempeh sports drink with pineapple juice, and P (3) = given tempeh sports drink with watermelon juice and pineapple juice).

Physical Exercise Protocols

Different physical activity protocols in the experimental animals were carried out during and after the adaptation period. During the adaptation period (7 days), the rats performed swimming exercises with very light intensity (10 minutes/day) each morning. After the adaptation period (during the study period), the rats performed intense acute swimming exercise. Rats were placed in a pool filled with water with a height of approximately 30 cm and left to swim for 30–40 minutes. The rats were then removed from the pool, and their fur was dried. Exercise was only performed once during the study period.

Administering the Tempeh-Based Sport Drink

The tempeh sports drink was given to the negative control group (K (-)) and the treatment groups (P (1), P (2), and P (3)), while the positive control group (K (+)) was given water (placebo) and no tempeh sports drink. A total of 5 mL of the tempeh sports drink was administered through one hour after the physical exercise.

Blood Sampling Protocol and Analysis of LDH Plasma Levels

Blood was drawn from the tail vein (vena coccygeal) with the rats restrained and without anesthesia (Kartika et al., 2014). Blood was drawn at a volume of 3 cc from each rat at two time points: 2 hours and 24 hours after administering the tempeh sports drink. The blood was then centrifuged to separate the plasma and serum. The analysis of plasma LDH levels in rats was carried out at the Pramita Clinical Laboratory in Semarang.

Data Analysis

All data were analyzed using SPSS 16 for Windows. Physicochemical characteristics of the tempeh sports drink and plasma LDH level data were analyzed quantitatively using one-way ANOVA analysis with a 95% confidence level. If the results of the analysis were significant, then Duncan's Multiple Range Test (DMRT) was performed to determine the real difference between the treatment means. In addition, data on plasma LDH levels were analyzed using a dependent t-test to determine differences between plasma LDH levels at 2 hours post-treatment and 24 hours post-treatment.

Ethical Clearance

All research procedures using experimental animals were in accordance with ethical protocols and obtained approval from the Ethics Commission of Semarang State University with letter number 132/KEPK/EC/2020.

Results

Physicochemical Characteristics of the Tempeh Sports Drink

This study formulated a tempeh-based sports drink combined with watermelon and/or pineapple juice. The sports drink product formulation analysis showed that there were significant differences in the content of BCAA, citrulline, protein, and antioxidant activity between formulations. It can be seen that there are differences in the levels of lysine ($p = 0.015$), isoleucine ($p = 0.0001$), and valine ($p = 0.0001$) between groups. In addition, there were differences in levels of citrulline ($p = 0.0001$), protein ($p = 0.0001$), and antioxidant ($p = 0.0001$) activity between groups.

Table 2. Differences in the content of branched-chain amino acids, citrulline, and antioxidants in sports drink products made from tempeh, which were formulated using various fruit juice compositions.

| Treatments (n = 12) | Parameters | | | | | |
|---------------------|---------------------------|----------------------------|---------------------------------|-------------------------------|-------------------------------|----------------------------|
| | Protein* (g) | Citrulline* (mg/100g) | Lysine* (mg/100 g) | Isoleucine* (mg/100 g) | Valine* (mg/100 g) | Antioxidant Activity* (%) |
| SPT1 | 3.87 ± 0.040 ^a | 6.09 ± 0.038 ^a | 2301.42 ± 126.275 ^{ab} | 1517.55 ± 71.672 ^a | 1546.53 ± 76.810 ^a | 2.22 ± 0.193 ^a |
| SPT2 | 4.54 ± 0.230 ^c | 14.60 ± 0.021 ^d | 2192.54 ± 48.592 ^a | 1527.13 ± 10.991 ^a | 1554.55 ± 9.081 ^a | 2.82 ± 0.550 ^{ab} |
| SPT3 | 4.23 ± 0.057 ^b | 12.24 ± 0.046 ^b | 2188.30 ± 150.681 ^a | 1750.33 ± 50.966 ^b | 1790.12 ± 51.105 ^b | 6.06 ± 0.976 ^c |
| SPT4 | 4.25 ± 0.072 ^b | 12.36 ± 0.042 ^c | 2445.08 ± 60.639 ^b | 1577.55 ± 26.510 ^a | 1616.58 ± 28.490 ^a | 3.81 ± 0.018 ^b |

All values are expressed as means ± SD (n = 12).

Tests of differences between means of treatments were conducted by one-way ANOVA, significant at $p < 0.05$; significance is denoted by *. Post hoc tests used Duncan's Multiple Range Test (DMRT), with significant differences between treatments denoted by superscript letters (a, b, c). Treatments (n = 12): SPT1, tempeh sports drink without fruit juice; SPT2, tempeh sports drink with watermelon juice; SPT3, tempeh sports drink with pineapple juice; and SPT4, tempeh sports drink with watermelon and pineapple juice.

Based on the results shown in Table 2, protein content in the SPT1 treatment differed significantly from the other treatments, although SPT 3 and SPT 4 treatments did not show significant differences in protein levels. On the other hand, the BCAA content of the SPT1 treatment did not differ significantly from other treatments, although the SPT3 and SPT4 treatments showed significant differences in BCAA content. The different formulations of sports drinks showed different contents of citrulline for each treatment. However, the antioxidant activity of the sports drinks showed a significant difference when SPT1 was compared with SPT3 and SPT4. The highest level of citrulline (14.60 ± 0.021 mg/100 g) was found in the SPT2 sports drink (combination of tempeh flour with watermelon juice). The highest antioxidant activity level was found in the SPT3 treatment ($6.06 \pm 0.976\%$).

Plasma LDH Levels in Rats

Based on the results of the study, plasma LDH levels 2 hours post-treatment were significantly higher compared with plasma LDH levels 24 hours post-treatment ($p = 0.0001$). The 24-hour post-treatment plasma LDH levels were lower. The difference in plasma LDH levels 2 hours and 24 hours after treatment were significantly different between the control group and the treatment group ($p = 0.006$); the rats given a tempeh-based sports drink with watermelon juice and pineapple juice (P (3)) showed a significant decrease in plasma LDH values compared with the control groups (K (+) and K (-)) and the other treatment groups (P (1) and P (2)). The changes in plasma LDH levels in male Wistar rats can be seen in Table 3.

Table 3. Changes in plasma LDH levels in male Wistar rats.

| Treatments | Plasma LDH level (IU/L) | | | p-value |
|------------|-------------------------|---------------------|------------------------|---------|
| | 2h | 24h | Δ^{\wedge} | |
| K (+) | 3355.2 ± 1229.42 | 787.0 ± 241.57 | 2567.2 ± 1288.46^a | 0.0001* |
| K (-) | 2022.4 ± 660.53 | 1107.2 ± 658.52 | 915.2 ± 460.49^a | |
| P (1) | 3349.8 ± 1711.23 | 863.2 ± 443.75 | 2486.6 ± 1970.96^a | |
| P (2) | 2197.6 ± 601.23 | 805.6 ± 573.91 | 1392.0 ± 669.24^a | |
| P (3) | 6745.4 ± 2827.81 | 1339.0 ± 657.01 | 5346.4 ± 2928.68^b | |

All values are expressed as means \pm SD (n = 30).

Difference mean test using paired t-test (comparison between means at 2 h and 24 h post-treatment), significant at $p < 0.05$

Significant showed with notation *

Δ^{\wedge} Difference of means test using one-way ANOVA (comparison among treatments and between control and treatment groups), significant at $p < 0.05$ ($p = 0.006$).

Treatments: K (+), positive control group given water (placebo); K (-), negative control group given tempeh-only sports drink (SPT1); P (1), treatment group given tempeh sports drink (SPT2); P (2), treatment group given tempeh sports drink (SPT3); and P (3), treatment group given tempeh sports drink (SPT4).

As shown in Table 3, the highest level of plasma LDH 2 hours post-treatment was found in P (3), which was given a tempeh-based sports drink with pineapple juice and watermelon juice, while the lowest level was found in K (-). The highest 24-hour post-treatment plasma LDH level was found in group P (3), which was given a tempeh sports drink with pineapple juice and watermelon juice, while the lowest level was found in group K (+). The highest decrease (Δ) plasma LDH level was shown by group P (3), while the lowest decrease was shown by group K (-).

Discussion

Physicochemical Characteristics of Tempeh Sports Drink

Tempeh is a BCAA-rich food, watermelon is a fruit high in citrulline, and pineapple contains bromelain, which is high in protease enzymes. The results show that the BCAA, citrulline, and antioxidant activity between sports drink formulations were significantly different. This can be attributed to the high BCAA levels in tempeh coupled with the high BCAA components in pineapple juice and watermelon juice. This is supported by research conducted in 2020, which examined the chemical composition of pineapple byproducts. The results of this study indicated that pineapple byproducts had a total protein content of $0.98 \pm 0.05\%$ w/w and a total free amino acid content of 38.5 mg amino acid/100 g (dry basis). The amino acid composition of pineapple byproducts comprises asparagine, serine, aspartic acid, glutamic acid, glutamine, cysteine, threonine, tyrosine, and valine (Campos et al., 2020). Another study conducted in 2019 that examined the nutritional content of watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) and egusi melon (*Citrullus colocynthis* (L.) Schrad.) showed that both watermelon and egusi contain amino acids, both essential amino acids (lysine, leucine, isoleucine, cysteine, methionine, phenylalanine, tyrosine, threonine, valine, and histidine) and nonessential amino acids (arginine, asparagine, glycine, glutamic acid, proline, and serine) (Falade et al., 2019). Therefore, a combination of the three components above should increase the total BCAA levels in the product. The highest levels of citrulline (14.60 ± 0.021 mg/100 g) were found in the SPT2 sports drink (combination of tempeh flour with watermelon juice). This is attributable to the high content of citrulline found in watermelon. Supported by previous research, watermelon has a higher citrulline content than any other citrulline source. Seedless yellow watermelon varieties have a higher citrulline content than other varieties. Based on gas chromatographic (GC-MS) analysis, 1 g of yellow watermelon contains 3.6 mg of citrulline (Rimando &

Perkins-Veazie, 2005). The addition of watermelon juice to a sports drink formulation made from tempeh flour can therefore increase the levels of citrulline in the end product. The highest antioxidant levels ($6.06 \pm 0.976\%$) were found in the SPT3 treatment. This is attributable to the phenolic compounds contained in pineapples that can be used as antioxidants. The phenolic compound content in pineapple fruit, 40.4 mg/100g GAE, is very beneficial to health (Lobo & Paul, 2017). In addition, pineapple contains vitamin C, which can also act as an antioxidant. A 100-g serving of pineapple fruit can fulfill 16.2% of the daily vitamin C requirement (Ozkanlar, 2012). Therefore, in the combination of tempeh flour and pineapple juice, the antioxidant content is expected to be higher than that in the other formulations. The findings above explain why the highest levels of citrulline were found in the SPT2 sports drink (combination of tempeh flour with watermelon juice) and the highest antioxidant content was found in the SPT3 treatment (combination of tempeh flour with pineapple juice).

Plasma LDH Levels in Rats

The results of this study also indicated a difference between LDH levels at 2 hours and 24 hours after administering the tempeh sports drink treatment. Initially, it appears that the LDH levels increased greatly, but 1 day after treatment, there was a significant decrease in LDH levels. This is thought to be the result of a combination of a longer resting and recovery period (more than 24 hours after training) and additional therapy in the form of a tempeh-based sports drink combined with watermelon and/or pineapple juice. The sports drink impacted the improvement of plasma LDH levels in experimental animals.

LDH enzyme is a tetrameric enzyme whose four subunits are present in two isoforms: H (in the heart) and M (in the muscle). LDH is also an intracellular enzyme that is widely distributed in tissues, especially in the heart, skeletal muscle, kidneys, and liver. LDH is needed to catalyze the change from pyruvic acid to lactic acid. LDH is released from damaged tissue, such as necrosis or changes in cell permeability. When the oxygen supply decreases, to meet the needs of ATP, the body converts aerobic metabolism to anaerobic, which is catalyzed by LDH (Rodwell et al., 2015; Lieberman et al., 2013).

BCAAs cannot be produced by the body and must come from food intake. These amino acids are metabolized mostly in skeletal muscles. Consuming BCAA-rich foods can stimulate muscle protein synthesis, prevent muscle protein breakdown, and reduce exercise-induced muscle damage, thereby delaying fatigue. The bromelain present in pineapples contains protease enzymes, which play a role in reducing exercise-induced muscle damage and inflammation, enhancing recovery. Meanwhile, consuming foods with citrulline content plays a role in improving athletes' performance. Several studies have suggested that citrulline can increase VO_2 max, delay muscle melt, and reduce post-workout muscle soreness. A 1-kg serving of watermelon can contain 2.1 mg of the amino acid citrulline (Sanz et al., 2017; Shing et al., 2016; Rizal & Segalita, 2018; Rimando & Perkins-Veazie, 2005).

This study is in line with previous research that states that watermelon juice has an effect on muscle fatigue and DOMS after weight training (Proia et al., 2016). Leucine, valine, and isoleucine BCAAs have an important role because they are responsible for energy metabolism in muscle work. Damage to muscles that occurs after exercise can speed up the recovery process with the help of BCAAs. BCAA supplementation carried out in previous studies had the effect of reducing muscle pain attacks and muscle damage that occurred after exercise (Jauhari et al., 2014; Shimomura et al., 2006). Several studies also stated that bromelain enzyme supplementation (a protease enzyme derivative from pineapple), combined with other proteolytic enzymes, can improve muscle function and reduce muscle pain (Miller et al., 2004; Buford et al., 2009; Beck et al., 2007).

Leucine is an amino acid that has anti-proteolytic properties and stimulates the protein synthesis process when combined with other amino acids (the BCAAs lysine, isoleucine, and valine). With this collaboration, protein synthesis can be further improved (Kirby et al., 2012). Other studies have also stated that BCAAs and leucine are both effective supplements in overcoming muscle damage. The intake of leucine can induce the regeneration of cell membranes damaged by exercise through the endogenous synthesis of HMB and its metabolites, namely HMG-CoA (Nicastro et al., 2014).

The results of this study contradict research conducted in 2018, which examined the effect of single or combined BCAA supplementation on DOMS and muscle damage due to resistance training. The study stated that LDH levels after 24 hours of BCAA supplementation showed a significant increase ($p = 0.0001$) compared to baseline LDH levels (265.49 ± 71.044 IU/L vs. $324.00 \pm 73,309$ IU/L) (Asjodi et al., 2018).

The content of citrulline in watermelon is a cofactor in the process of forming nitric oxide (NO). NO plays a role in increasing vasodilation, which can accelerate the entry of nutrients into the blood, thus speeding up the recovery process. In addition, the excretion of ammonia and lactic acid can be accelerated. It is this accumulation of lactic acid and ammonia that causes muscle fatigue. With reduced levels of lactic acid and ammonia in the body, the muscles take longer to reach muscle fatigue. Thus, the effort that can be generated by the muscles increases, and more repetitions can be accomplished.

Conclusions

Based on the study results, it can be concluded that the highest protein and citrulline content was found in the tempeh-based sports drink that with watermelon juice (SPT2). Meanwhile, the tempeh-based sports drink that showed the highest BCAA content and antioxidant activity contained pineapple juice (SPT3). In addition,

administering a tempeh-based sports drink that contained watermelon juice or pineapple juice significantly reduced LDH levels in rat plasma at 24 hours post-treatment. Administering the tempeh-based sports drink that contained watermelon juice and pineapple juice (SPT4) provided the highest and the most significant reduction in LDH levels when compared with the control group and other treatment groups. In the other words, administering 600 mL of a tempeh-based sports drink that contained watermelon juice and pineapple juice (containing 4.25 ± 0.072 g protein, 12.36 ± 0.042 mg citrulline, 2445.08 ± 60.639 mg lysine, 1577.55 ± 26.510 mg isoleucine, 1616.58 ± 28.490 mg valine, and $3.81 \pm 0.018\%$ of antioxidant activity) can reduce LDH levels as much as 5346.4 ± 2928.68 IU/L 24 hours post-treatment.

One limitation of this research is that because this study used a post-test-only control group design, it does not provide baseline data. However, to improve the quality of the results, researchers recorded post-treatment data twice: 2 hours and 24 hours after treatment. The study provides details for developing and implications of administering a tempeh-based sports drink that can be used to improve the metabolic profile of muscle injury after training. In addition, this study indicates a need to identify the effect of sports drinks on the metabolic changes of athletes.

This study can be used as the basis for further research on the effects of sports drinks on the post-workout recovery process. Furthermore, future research can more deeply explore the effects of sports drinks on other muscle injury indicators to determine proper nutritional treatment for post-training injury management.

References:

- Asjodi, F., Khotbesara, R. D., Gargari, B. P., Izadi, A. (2018). Impacts of combined or single supplementation of branched-chain amino acids on delayed onset muscle soreness and muscle damage following resistance exercise. *Progress in Nutrition*, 20(2), 263-272.
- Beck, T. W., Housh, T. J., Johnson, G. O., Schmidt, R. J., Housh, D. J., Coburn, J. W., et al. (2007). Effects of a protease supplement on eccentric exercise-induced markers of delayed-onset muscle soreness and muscle damage. *J Strength Cond Res.*, 21(3), 661–667.
- Buford, T. W., Cooke, M. B., Redd, L. L., Hudson, G. M., Shelmadine, B. D., Willoughby, D. S. (2009). Protease supplementation improves muscle function after eccentric exercise. *Med Sci Sports Exerc*, 41(10), 1908–1914.
- Campos, D. A., Ribeiro, T. B., Teixeira, J. A., Pastrana, L. P. M. (2020). Integral valorization of pineapple (*Ananas comosus* L.) by-products through a green chemistry approach towards added value ingredients. *Foods*, 9(60), 1–22.
- Chandanwale, A., Langade, D., Sonawane, D., Gavai, P. (2017). A randomized, clinical trial to evaluate efficacy and tolerability of trypsin:chymotrypsin as compared to serratiopeptidase and trypsin:bromelain:rutoside in wound management. *Advances in Therapy*, 290, 148-156.
- Falade, O., Otemuwiya, I. O., Adekunle, A. S., Adewusi, S. A. O. O. (2019). Nutrient composition of watermelon (*Citrullis lanatus* (Thunb.)Matsum.&Nakai) and egusi melon (*Citrullus colocynthis* (L.)Schrad.) seeds. *Agric. Conspec. Sci.*, 85, 43–49.
- Hulmi, J. J., Lockwood, C. M., Stout, J. R. (2010). Review effect of protein/essential aminoacids and resistance training on skeletal muscle hypertrophy: A case for whey protein. *Nutr Metab.*, 7, 51.
- Jauhari, M., Sulaeman, A., Riyadi, H., & Ekayati, I. (2014). Development of tempeh-based sports drinks for muscle damage recovery. *Journal of AGRITECH*, 34(3), 285-290.
- Kartika, D., Dewi, F. N. A., Iskandriati, D., Winoto, I., Permanawati, Narani, A., Suhartin, Budiarsa, I. N. (2014). Hematological parameter values of Sprague Dawley rats and BALB/C mice in Indonesia as a reference in biomedical research. Proceedings of the 13th National Veterinary Scientific Conference (KIVNAS). Palembang 23-26 November 2014.
- Kirby, T. J., Triplett, N. T., Haines, T. L., Skinner, J. W., Fairbrother, K. R., McBride, J. M. (2012). Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. *Amino acids*, 42(5), 1987-1996.
- Laumonier, T., Menetrey, J. (2016). Muscle injuries and strategies for improving their repair. *Journal Of Experimental Orthopaedics*. 3:15, 1-9.
- Lieberman, M., Marks, A. D., Peet, A. (2013). Marks basic medical biochemistry: a clinical approach. Fourth Edition. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.
- Lobo, M. G., Paull, R. (2017). Handbook of Pineapple Technology, USA: John Wiley & Sons, Ltd.
- Mameli, A., Natoli, V., Casu, C. (2020). Bromelain: an overview of application in medicine and dentistry. *Biointerface Research in Applied Chemistry*, 11(1), 8165-8170.
- Margaretta, D. L., Chow, A., Dirgantara, Y., Sadono, M., Sandra, F. (2015). Macerated-pineapple core crude extract-derived bromelain has low cytotoxic effect in NIH-3T3 fibroblast. *Indones Biomed J.*, 7(2), 101-106.
- Marvin, T., Lorkowski, G., Reule, C., Rau, S., Pabst, E., Vester, J. C., Pabst, H. (2017). Effects of a systemic enzyme therapy in health active adults after exhaustive eccentric exercise: a randomised, two-stage, double-blinded, placebo-controlled trial. *BMJ Open Sport Exerc. Med.*, 2, 1-10.
- Meambarbashi, A. (2017). Herbs and natural supplements in the prevention and treatment of delayed-onset muscle soreness. *Avicenna J Phytomed.*, 7(1), 16-26.

- Miller, P. C., Bailey, S. P., Barnes, M. E., Derr, S. J., Hall, E. E. (2004). The effects of protease supplementation on skeletal muscle function and DOMS following downhill running. *J Sports Sci.*, 22(4), 365–72.
- Mueller-Wohlfahrt, H. W., Haensel, L., Mithoefer, K., Ekstrand, J., English, B., McNally, S., et al. (2013). Terminology and classification of muscle injuries in sport: the Munich consensus statement. *BJSM*, 47(6), 342-350.
- Nicastro, H., Carnauba, R. A., Massunaga, N. D., da Fonseca, A. B. B., Paschoa, I. V., Naves, A., et al. (2014). Are the BCAAs/leucine supplementation effects on exercise-induced muscle damage related immunity response or to Hmβ. *J Nutrition Health Food Sci.*, 2(2), 1-3
- Nicastro, H., da Luz, C. R., Chaves, D. F. S., Bechara, L. R. G., Voltarelli, V. A., Rogero, M. M., et al. (2012). Does branched-chain amino acids supplementation modulate skeletal muscle remodeling through inflammation modulation? Possible mechanisms of action. *J Nutr Metab.*, 2012, 1-10.
- Owens, D., Twist, C., Copley, J., Howatson, G., Close, G. (2019). Exercise-induced muscle damage: what is it, what cause it and what are the nutrition solutions?. *European Journal of Sport Science*, 19, 71-85.
- Ozkanlar, S. F. A. (2012) Antioxidant vitamins in atherosclerosis – animal experiments and clinical studies. *Adv Clin Exp Med.*, 21(1), 115–123.
- Proia, P., Di Liegro, C. M., Schiera, G., Fricano, A., Di Liegro, I. (2016). Lactate as a metabolite and a regulator in the central nervous system. *Int. J. Mol. Sci.*, 17, 1–20.
- Rahmi, Leila, S, Mursyid, Wulansari, D., (2018), Spiced tempeh formulation and nutritional content testing. *Journal of Agroindustrial Technology and Management*, 7(1), 57-65.
- Raimi F., Jawis, M., Hashim, H., Zainuddin, Z. (2020). Single one-off dose of nigella sativa does not attenuate indirect markers of exercise-induced muscle damage as a mode of inflammation. *Journal of Physical Education and Sport (JPES)*, 20(3), 1444-1454.
- Rhim, H. C., Kim, S. J., Park, J., Jang, K. (2020). Effect of citrulline on post-exercise rating of perceived exertion, muscle soreness, and blood lactate levels: A systematic review and meta-analysis. *Journal of Sport and Health Science*, 9(6), 553-561.
- Rimando, A. M., Perkins-Veazie, P. M. (2005). Determination of citrulline in watermelon rind, *J. Chromatogr. A*, 107, 196–200.
- Riyanto, B., Trilaksani, W., Lestari, R. (2016). Octopus protein hydrolyzate-based sports nutrition drink. *JPHPI*, 19(3), 339-47.
- Rizal, M., Segalita, C. (2018). The role of the amino acid citrulline in improving sports performance in athletes. *Amerta Nutr.*, 2(4), 299-306.
- Rodwell, V. W., Bender, D. A., Botham, K. M., Kennelly, P. J., Weil, P. A. (2015). Harper’s Illustrated Biochemistry: 30th Edition, New York: Mcgraw-Hill Education.
- Sanz, J. M. M., Norte, A., Garcia, E. S., Sospedra, I. (2017). Sustained energy for enhanced human functions and activity: Chapter 22. Branched amino acids and sports nutrition and energy homeostasis. Academic Press: 351-362.
- Schulz, A., Fuchs, P. C., Oplaender, C., Valdez, L. B., Schiefer, J. L. (2018). Effect of bromelain-based enzymatic debridement on skin cells. *Journal of Burn Care & Research*, 39, 527-535.
- Shenoy, S., Dhawan, M., Sandhu, J. S. (2017). Effect of Chronic Supplementation of Branched Chain Amino Acids on Exercise-Induced Muscle Damage Trained Athletes. *Journal of Sports Science*, 5, 265-273.
- Shimomura, Y., Yamamoto, Y., Bajotto, G., Sato, J., Murakami, T., Shi-momura, N., Kobayashi, H., Mawatari, K. (2006). Nutraceutical effects of branched-chain amino acids on skeletal muscle. *J Nutr.*, 136, 529S-532S.
- Shing, S. M., Chong, S., Driller, M. W., Fell, J. W. (2016). Acute protease supplementation effects on muscle damage and recovery across consecutive days of cycle racing. *Eur J Sport Sci.*, 16(2), 206-212.
- Sirait, P. A., Abrori, C., Suswati, E. (2016). The effect of watermelon juice on muscle fatigue and delayed onset muscle soreness after weight training. *Pustaka Kesehatan*, 4(1), 132-135.
- Tang, J. E, Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A., Phillips, S. M. (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol.*, 107(3), 987–92.
- Tartibian, B., Maleki, B. H., Abbasi, A. (2009). The effects of ingestion of omega-3 fatty acids on perceived pain and external symptoms of delayed onset muscle soreness in untrained me. *Clin J Sport Med.*, 19, 115-119.
- Tipton, K., D. (2015). Nutritional Support for Exercise-Induced Injuries. *Sports Med.*, 45, 93–104.