

# Effects of Watermelon Juice and Pure L-citrulline on Muscle Soreness after Eccentric Exercise: A Comparative Study

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## Effects of Watermelon Juice and Pure L-citrulline on Muscle Soreness after Eccentric Exercise: A Comparative Study

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### Abstract

Muscle overuse during exercise increases the risk of muscle injury. L-citrulline, one of the ergogenic substances highly found in watermelon, is frequently used to reduce muscle soreness. This study aims to compare the effects of pure L-citrulline and watermelon juice in reducing delayed-onset muscle soreness (DOMS) and muscle damage using uric acid as a marker for inflammation. A single-blind controlled trial was employed among 33 participants selected with a purposive sampling method. The participants were given either a mineral water drink containing 1.2 g of L-citrulline supplementation, 750 ml of watermelon juice, or a bottle of mineral water as a placebo administered h-2 before exercising. A multiple-sprint with a deceleration phase was used to induce DOMS. There was a significant decrease of DOMS 12h and 24h post-exercise with watermelon juice and pure L-citrulline ( $p=0.001$ ). However, there was no significant difference in effectiveness between both forms of supplementation ( $p=0.748$ ). There were no significant changes in uric acid levels post-exercise with both forms of supplementation. Both watermelon juice and pure L-citrulline show similar effectiveness in improving subjective feelings of DOMS but fail to provide evidence of their ability to reduce muscle inflammation significantly via the reduction of uric acid levels.

**Keywords:** Citrulline; delayed-onset muscle soreness; muscle inflammation; uric acid; watermelon juice

### 1. Introduction

Eccentric exercise is frequently employed in diverse sporting activities due to its numerous advantages, such as optimizing muscle strength and physical coordination (Hody et al., 2019; Vogt & Hoppeler, 2014). The amount of energy required for eccentric contraction is lower than any other type of contraction, yet it generates a greater force (Hody et al., 2019). Although it has many advantages, eccentric exercise increases the risk of muscle injury. Delayed-onset muscle soreness (DOMS) is one of the manifestations that can emerge as a result of muscle injury (Rusdiawan et al., 2020). DOMS frequently develops between 12 and 24 hours after exercise, reaching its highest intensity between 24 and 72 hours, and resolves spontaneously within 5 to 7 days. Even though it may resolve by itself, the sensation of muscle pain may discourage ordinary people from initiating a new physical routine and disrupt athletes' rehabilitation and training programs (Zulaini et al., 2021).

Excessive muscle use may also increase uric acid levels released from damaged cells (Yoshida et al., 2019). Uric acid induces inflammation by activating the NF- $\kappa$ B signaling pathway. Therefore, it can be used as one of the inflammatory biomarkers (Spiga et al., 2017). Otherwise, further inflammation can enhance uric acid production, continually affecting each other like a vicious cycle (Suzuki et al., 2022). The elevation of uric acid concentrations is also associated with the enzymatic activity of xanthine oxidase, which is observed to increase within 12 hours following eccentric exercise (Retamoso et al., 2016). Within 24 hours after eccentric training, elevated uric acid and DOMS were also identified in athletes (Chatznikolaou et al., 2014). High-intensity exercise significantly increases

the activity of the purine metabolic pathway. This process releases inflammatory mediators by enhancing the formation of reactive oxygen species (ROS). In response, nociceptor sensitization occurs, resulting in DOMS (Rajakumar et al., 2013; Yoshida et al., 2019).

To minimize the adverse impacts of physical exercise, optimize muscular effort, and enhance athletic performance, one potential alternative approach is the consumption of ergogenic substances, such as through supplementation (Gusmaya et al., 2019). One frequently used dietary supplement in the sporting field is L-citrulline (Huerta Ojeda et al., 2019). L-citrulline is an endogenous amino acid infrequently present in food but abundant in watermelon (Allerton et al., 2018). L-citrulline is converted to L-arginine in the kidneys, serving as a nitric oxide synthase (NOS) substrate. Nitric oxide (NO) serves multiple purposes, including facilitating muscle repair via the activation of satellite cells and the secretion of myotropic factors; therefore, it might alleviate muscle pain (Rhim et al., 2020). Additionally, L-citrulline significantly inhibits the xanthine oxidase (XO) enzyme, preventing the formation of uric acid by impeding the xanthine degradation process (Ozcan et al., 2022; Yoshida et al., 2019).

Various forms of L-citrulline supplementation have been established, including watermelon juice, pure L-citrulline, and citrulline malate (CM) (Rhim et al., 2020). Research conducted by Tarazona-Díaz et al. (2013) demonstrated that watermelon juice consumption reduced muscle pain. Another study discovered oral L-citrulline could significantly reduce DOMS (Suzuki et al., 2016). Since watermelon juice is readily available in the environment, researchers are interested in comparing it to pure L-citrulline to use it as a determining factor in selection. Comparative studies examining the effectiveness of different forms of L-citrulline supplementation in reducing DOMS with the use of uric acid as an indicator of muscle inflammation remain scarce. Hence, researchers are intrigued and aim to compare the effectiveness of watermelon juice and pure L-citrulline in reducing delayed-onset muscle soreness (DOMS) and muscle damage using uric acid as an inflammation marker.

## 2. Method

This research used pre-experimental quantitative methods with a single-blind study design. It was conducted at colleges dormitories in Depok, West Java, Indonesia. The dormitories were selected as the research site because they implemented a quarantine system that helps to reduce the likelihood of dropouts while easing data collection. In addition, boarding colleges enforce a standardized food consumption approach, allowing greater control over influencing variables.

The subjects used for the study were members of the population who met the criteria established by the researchers. The inclusion criteria for the study were based on the following: male between the ages of 18 and 25, normal body mass index (BMI) (18.5 to 24.9 kg/m<sup>2</sup>), normal uric acid levels (3.5 to 7.2 mg/dL), willing to abstain from consuming coffee and alcohol for 24 hours before treatment, refrain from performing intense physical activity for 48 hours before treatment. Participants who had a musculoskeletal disease or injury within the six months before treatment, were following a specific diet program, or had consumed supplements in the past year (as determined by a pre-study questionnaire) were excluded from the study. Participants were instructed not to consume additional foods or supplements the day before and throughout the research. They were required to consume food provided by the research site.

The sampling technique used a non-probability method, precisely the purposive sampling method. All populations that matched the specified criteria for inclusion can be incorporated into the research sample. According to Roscoe (1975), a sample size of 10 to 20 individuals per group is necessary to perform a simple experimental study. Three sample groups were used in this study; therefore, a

minimum of thirty samples were required. To prevent dropouts, the overall sample size was augmented by 10%. Hence, a sample size of 33 was used for this study.

This study used pure L-citrulline and watermelon juice as a form of L-citrulline supplementation. Tarazona-Díaz et al. (2013) discovered that an acute administration of 1.17 g L-citrulline was adequate to reduce DOMS, while larger L-citrulline doses did not result in a statistically significant difference in muscle soreness. To round up, the L-citrulline dose used in this investigation was 1.2 g.

The following forms of supplements were distributed to respondents: (1) 330 mL of mineral water containing 1.2 g of L-citrulline, (2) 750 mL of watermelon juice, or (3) 330 mL of mineral water as a placebo. Despite employing a single-blind design, watermelon juice might exhibit color and flavor variations compared to the L-citrulline solution and placebo. However, the L-citrulline solution and the placebo appeared to be identical. Participants who were administered the placebo were not informed of the absence of the supplement.

The powdered form of L-citrulline manufactured by Doctor's Best, Inc., located in California, USA, was used in this study. 1.2 g of L-citrulline powder was measured using a microdigital scale and diluted with 330 ml of mineral water. The L-citrulline powder does not alter the color or form of water, making it indistinguishable from mineral water without L-citrulline. Watermelon juice was made using red seedless watermelon (*Citrullus lanatus*), originated from Banyuwangi, East Java, Indonesia, and purchased at a nearby retailer. To provide 1.2 grams of L-citrulline, approximately 314 to 678 grams of watermelon would be sufficient (Allerton et al., 2018; Davis et al., 2011). According to the median calculation, 500 grams of watermelon flesh were mixed with 200 ml of water to make juice. Therefore, an estimated volume of 750 ml of watermelon juice might be produced. Afterwards, the juice was placed into beverage containers to make it more convenient to consume. The supplement and placebo were consumed two hours before exercise. The highest concentration of plasma L-citrulline was achieved about 40-60 minutes after consumption, whereas the maximum concentration of plasma arginine was reached approximately two hours after consumption. This gap was related to the conversion process from citrulline to arginine (Gonzales & Trexler, 2020).

This research employed multiple sprints as the eccentric exercise. Warm-ups were performed before exercise to prevent injury. Multiple sprints of 40 x 15 meters were performed, followed by a 5-meter deceleration zone, which was shown to cause exercise-induced muscle damage (EIMD). Sprinting was done at maximum velocity. Participants decelerated to the limit of the deceleration zone before coming to a complete stop (Sanjaya et al., 2021; Wooley et al., 2014).

Muscle soreness was measured 12 and 24 hours after exercise, and uric acid levels were measured right before exercise and 12 and 24 hours after exercise. Uric acid levels were measured immediately before exercise to avoid influencing the measurement results. The visual analog scale (VAS) was employed to quantify DOMS. The muscular contraction and relaxation protocol were performed actively and passively. The participants reported pain levels on a 10-centimeter line (Lazaridou et al., 2018). The plot on the line was measured with a single-digit approach following the comma.

The research received approval from Dr. Moewardi General Hospital's Health Research Ethics Committee, with the following Ethical Clearance No. 1144/VII/HREC/2023. All participants were provided with a comprehensive explanation of the research, which was also detailed in the pre-research explanation document (PSP) distributed to them. Those who agreed to participate in this study filled out and signed an informed consent form voluntarily, without compulsion.

Before doing bivariate analysis, the normality of the data was assessed using the Shapiro-Wilk due to a small number of samples (<100). If the data distribution is normal, a one-way ANOVA test was used to assess the relationship between L-citrulline supplementation and DOMS, as well as the relationship between L-citrulline supplementation and uric acid levels. If the results were significant, post-hoc tests were performed using the Tukey test to assess the differences between groups. A non-parametric test, known as the Kruskal-Wallis test, was performed if the data distribution was abnormal. If the results were significant, pairwise comparisons can identify differences across each group pair. A paired test was also conducted to compare the pre-test and post-test values of the uric acid level data. A paired sample T-test was conducted if the data had a normal distribution. In the case of abnormal distribution of data, the Wilcoxon test was employed as a non-parametric alternative.

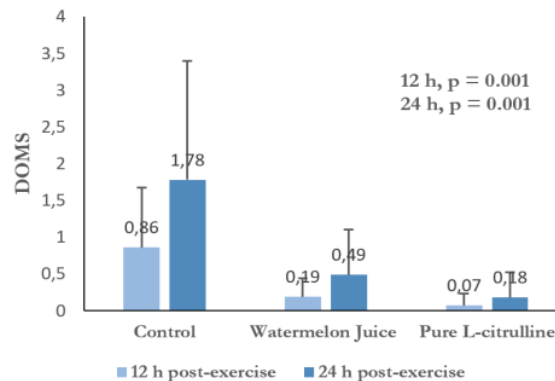
### 3. Result

The distinctions in characteristics among the groups of respondents were not significantly divergent.

**Table 1. Participants' characteristic**

Characteristics	Groups		
	Control	Watermelon Juice	Pure L-citrulline
Age			
Mean ± SD	20 ± 1.27	19.09 ± 1.04	19.73 ± 1.56
Minimum Range	19	18	18
Maximum Range	22	21	22
BMI			
Mean ± SD	21.79 ± 1.81	21.22 ± 2.09	21.22 ± 2.02
Minimum Range	19.10	18.69	18.69
Maximum Range	24.80	24.80	24.60

Table 1 shows that each group's average age and BMI were generally similar. Additionally, figure 1 revealed no notable variations in the pre-exercise uric acid levels among the participants in the three groups. The similarity of these traits indicates that the study began in the same setting for each group.



**Figure 1. DOMS 12-hour and 24-hour post-exercise**

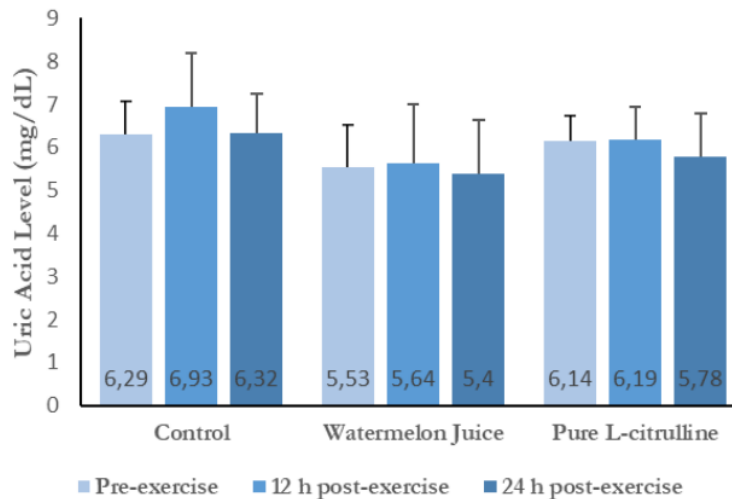
According to the graph shown in Figure 1, the control group had the highest mean of DOMS 12 hours and 24 hours after exercise, followed by the watermelon juice and pure L-citrulline groups. The analysis of differences between the three groups at 12 and 24 hours after exercise revealed a significant difference ( $p < 0.05$ ).



**Table 2. Pairwise comparison of DOMS values between each group**

DOMS values using VAS	P-values of group pairs		
	Control– Watermelon Juice	Control–Pure L-citrulline	Watermelon Juice– Pure L-citrulline
12 h post-exercise	0.039	0.001	0.748
24 h post-exercise	0.047	0.001	0.761

Nevertheless, upon examining the comparisons between each pair of groups, as shown in Table 2, it was observed that there were no significant differences ( $p > 0.05$ ) between the watermelon juice and pure L-citrulline groups 12- or 24-hours following exercise.



**Figure 2. DOMS 12-Hour and 24-Hour Post-Exercise**

As shown in Figure 2, the mean values of uric acid levels increased 12 hours after exercise in all three groups. The largest rise was observed in the control group. After 24 hours, uric acid levels in the group given watermelon juice and pure L-citrulline had reduced, but those in the control group still had an increase.

**Table 3. Analysis of uric acid changes post-exercise**

Changes ( $\Delta$ ) of uric acid levels	p-values
12 h post-exercise	0.463
24 h post-exercise	0.728

Upon analyzing uric acid level changes ( $\Delta$ ) 12- and 24-hours following exercise, no statistically significant differences were observed among the three groups as mentioned in Table 3. The analysis of differences in pre- and post-test uric acid levels provided similar results ( $p > 0.05$ ).

**4. Discussion**

The study's findings show a significant decrease in delayed-onset muscle soreness (DOMS) experienced 12 and 24 hours following eccentric exercise when supplemented with L-citrulline. However, there was no difference in effectiveness between the two forms of L-citrulline supplementation employed in this study: watermelon juice and pure L-citrulline solution. At the same dose, both are generally beneficial in reducing post-exercise DOMS.

Multiple hypotheses exist regarding the etiology of DOMS; these include tissue injury, inflammation, lactate release, muscle spasms, and the production of oxidative stress (Hody et al., 2019). L-citrulline has been found to have an antioxidant effect and play a role in the inflammatory process, which can help prevent skeletal muscle damage (Ghozali et al., 2023). It is believed that L-citrulline decreases DOMS by increasing ammonia clearance and inhibiting NO synthesis (Rhim et al., 2020). Long-term L-arginine administration enhances NO bioavailability (Smeets et al., 2022). A single dosage of L-citrulline can also increase NO bioavailability (Theodorou et al., 2021). As it bypasses the first pass of hepatic metabolism, L-citrulline, a precursor to L-arginine, is regarded as a more efficient precursor for NO synthesis (Rashid et al., 2020). NO synthesis facilitates the recovery process subsequent to exercise by stimulating satellite cells involved in muscle regeneration (Gonzales & Trexler, 2020; Rhim et al., 2020).

To date, there is a lack of research comparing the effectiveness of various forms of L-citrulline supplementation. However, the findings of this study were consistent with the results reported by Martinez-Sanchez et al. (2017), who found that administering L-citrulline supplementation in the form of watermelon juice 24 hours after the half-marathon race resulted in a significant decrease in the sensation of muscle soreness. Aside from that, another study using 500 mL of watermelon juice with the premise that it contained 1.17 g of L-citrulline found a significant decrease in lactic acid levels when athletes consumed watermelon juice. Lactic acid was used as a marker of muscle exhaustion (Arimbi et al., 2022; Permadi et al., 2023). In contrast to the findings above, acute administration of 335 ml of watermelon juice did not affect muscle soreness 24 hours following exercise (Blohm et al., 2020).

Aside from watermelon juice supplementation, it was discovered that ingesting 3 g of L-citrulline over seven days can lower perceived exertion and boost blood NO levels (Terasawa & Nakada, 2019). Oral L-citrulline at 2.4 g for seven days can also significantly enhance VAS scores compared to the placebo group. According to Suzuki et al. (2016), a single dose of L-citrulline is insufficient to improve athletic performance. On the other hand, Rhim et al. (2020) concluded in their meta-analysis that acute L-citrulline supplementation in any form, even at a single dose, can significantly alleviate muscle soreness 24 hours post-exercise.

In addition, malate has been used in another form of L-citrulline supplementation. It has been observed that malate can enhance ATP availability (Agudelo et al., 2019). Acute citrulline malate administration may reduce muscle fatigue and increase muscle endurance during exercise (Vårvik et al., 2021). However, there is insufficient evidence of its effect on muscular recovery function (Gough et al., 2021). Muscle fatigue and post-exercise recovery are unaffected by the acute administration of 3 and 6 grams of citrulline malate (Bezuglov et al., 2022).

Uric acid was used as a marker for muscle inflammation in this study. There was a positive correlation between elevated uric acid levels and musculoskeletal complaints (Reddy et al., 2021). Excessive muscle contraction increases uric acid production, contributing to the inflammatory process (K. Suzuki et al., 2022). This study's findings indicated no statistically significant differences in uric acid levels post-exercise among the three groups. So far, there has been relatively limited research on the effects of L-citrulline supplementation on uric acid levels. Nevertheless, the results of this study align

with the research by Martinez-Sanchez et al. (2017), who observed no significant differences in uric acid levels between the group that consumed watermelon juice and the placebo group.

Although the difference was not statistically significant, L-citrulline supplementation decreased the mean of change in uric acid levels. Ischemia-induced muscle conditions during exercise can stimulate purine metabolism, forming uric acid as the end product (Yoshida et al., 2019). Xanthine oxidase inhibitors have been found to effectively reduce muscle pain resulting from excessive exercise by blocking the production of uric acid (Chen et al., 2021). Under intermittent hypoxic conditions, L-citrulline can inhibit the xanthine oxidase (XO) enzyme. The absence of xanthine degradation results in the non-formation of uric acid and reactive oxygen species (ROS), contributing to muscle inflammation (Ozcan et al., 2022; Yoshida et al., 2019). Uric acid also stimulates inflammation through the NF- $\kappa$ B pathway by triggering TLR4 and increasing NF- $\kappa$ B transcription (Wang et al., 2022). Administration of L-citrulline has been shown to effectively decrease NF- $\kappa$ B expression, thereby mitigating one of the contributors to inflammatory processes (Ba et al., 2022; Darabi et al., 2019; Ghozali et al., 2023).

The effect of L-citrulline supplementation on uric acid levels may not be significant due to a dosage below the optimal level required to influence the xanthine oxidase enzyme. L-citrulline can be biosynthesized in several ways, including its involvement in the NO cycle. In the NO cycle, L-citrulline serves as a precursor for arginine synthesis, a substrate for the enzyme nitric oxide synthase (NOS) (Aguayo et al., 2021). Unlike its method for lowering muscle pain through NO production, L-citrulline metabolism does not directly engage XO. Therefore, a longer process or higher dose may be required (Ozcan et al., 2022). According to Gonzalez & Trexler (2020), 3 g is the minimum effective dose consumed daily.

## 5. Conclusion and Recommendation

Administering various forms of L-citrulline supplementation at the same dosage might result in similar effectiveness in reducing delayed-onset muscle soreness (DOMS). To achieve a higher dose of L-citrulline, watermelon juice must be consumed in large doses, which can be challenging to do simultaneously. Unlike watermelon juice, pure L-citrullin comes in a powdered form and can be easily added to any beverage, allowing for the effortless consumption of larger doses. This can be a determining factor in the selection of the supplementation form. Due to the likelihood that L-citrulline metabolism does not involve xanthine oxidase directly, this study is unable to demonstrate that L-citrulline reduces muscle inflammation by decreasing uric acid levels. Additional research is necessary to explore alternative inflammatory markers that are linked to the development of DOMS, as relying solely on subjective perception through visual analog scale (VAS) assessments may not provide a comprehensive understanding.

## References



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