ORIGINAL ARTICLE

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Effects of Beetroot and Pomegranate Juice Supplementation on Creatine Kinase and C-Reactive Protein in Amateur Soccer Players

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ABSTRACT

Background: Intense physical activity, such as football (soccer), can induce exercise-induced muscle damage (EIMD) and associated inflammatory responses. These perturbations can lead to increased levels in serum biomarkers such as creatine kinase (CK) and C-reactive protein (CRP). In response, athletes increasingly employ various recovery modalities, with nutritional interventions featuring antioxidant properties representing a prominent strategy.

Aims: This study aimed to evaluate and compare the efficacy of beetroot juice (BEJ) and pomegranate juice (POJ) beverages recognized for their antioxidant and anti-inflammatory potential, on modulating CK and CRP levels in football players throughout a regular training cycle.

Participants and Methods: A cohort of 18 amateur football players was allocated into three groups (n=6 per group): a pomegranate juice (POJ) group; a beetroot juice (BEJ) group; and a placebo (PLA) group. Baseline CK and CRP levels were determined before and after a high intensity training (HIT) session prior to supplementation. Participants then consumed their daily supplement for 22 days. CK and CRP levels were re-assessed 24 hours before and after HIT sessions on days 12 and 22 of the intervention.

Results: Following the 22-day supplementation period, CK levels was significantly lower in both POJ (209.30 ± 44.25 IU/L, $p < 0.0001$) and BEJ (188.27 ± 26.29 IU/L $p < 0.0001$) compared to the PLA group (327.75 ± 15.32 IU/L). No statistically significant difference in CK reduction was observed between the POJ and BEJ groups ($p > 0.05$). In contrast, CRP levels did not exhibit any significant changes across the groups at any measurement point ($p > 0.05$).

Conclusions: The findings reveal that both pomegranate and beet juices supplementation offer comparable protective effects against EMID, as demonstrated by the attenuation of post-exercise CK levels. This underlines the significance of the quality and diversity of bioactive compounds in nutritional recovery strategies. The study also highlights the complex relationship between exercise, muscle damage, and systemic inflammation, necessitating the exploration of further markers for a complete assessment.

Keywords: *Beta vulgaris*; *Punica granatum*; Antioxidant supplementation; Exercise-induced muscle damage (EIMD); C-reactive protein; Team sports nutrition; Recovery strategies.

Article Information

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1 INTRODUCTION

The competitive soccer is organized around weekly microcycles of training, tapering, competition, and recovery. However, the stress of frequent matches and training sessions increases the risk of injury and performance decline due to accumulated fatigue, muscle damage, and inflammation (Ispirlidis *et al.*, 2008). Studies involving professional players demonstrated that such activities can lead to acute rise in muscle damage markers such as creatine kinase (CK) and inflammatory markers like C-reactive protein (CRP). the Restoration of CK and CRP concentrations to baseline levels is often prolonged over several weeks, thereby impeding complete physiological recovery (Becker *et al.*, 2020). Consequently, a recovery window exceeding 72 hours is frequently necessary for players to re-establish pre-match performance capacity, a period during which the resolution of muscle damage and inflammation is ongoing (Daab *et al.*, 2021).

Recovery, within the domain of exercise physiology, constitutes a complex, multifactorial process involving the restoration of physiological and performance parameters to baseline levels following exercise-induced disruptions (Silva

& Macedo, 2011). This process includes the normalization of biochemical markers indicative of muscle damage, such as CK, the attenuation of inflammatory responses, including CRP levels, the restoration of muscle function, and the return of performance capacity to pre-exercise levels (Barnett, 2006; Brancaccio *et al.*, 2010; Halson, 2014; Kellmann, 2010; Peake *et al.*, 2017).

In contemporary athletic practice, players in team sports routinely employ a variety of recovery methods subsequent to training and competition. Among these, nutritional interventions, widely recognized and easily accessible approaches for promoting the recovery of performance and physiological disruptions following engaging in soccer-specific physical activity (Ranchordas *et al.*, 2017). Available research suggests that dietary antioxidants and phytochemical supplements can reduce exercise-induced muscle damage symptoms. These benefits include decreased soreness, preserved muscle function, and reduced inflammation, particularly following eccentric exercise protocols. (Clifford, Bell, *et al.*, 2016a; Pereira Panza *et al.*, 2015).

The growing importance of plant-derived antioxidants has grown been amplified in response to growing prominence of adverse effects associated with synthetic antioxidants (Zeng *et al.*, 2020). Consequently, scientists and consumers interest has increasingly pivoted towards healthy nutrition, natural food sources, and additive-free antioxidants, especially those abundant in fruits and vegetables. Beetroot (*Beta vulgaris*) and pomegranate (*Punica granatum*) juices, renowned for their abundance of polyphenols, have been employed as supplements in various domains, including sports. A review by Harty *et al.* (2019) have demonstrated that consumption of beetroot juice or pomegranate juice may have beneficial effects on exercise-induced muscle damage. However, a consensus on their efficacy remains discussed, and there is also little research on nutritional or supplementation approaches in real post-match soccer environments (Kotsis *et al.*, 2018) or in the context of real soccer microcycles utilizing these juices. To the authors' knowledge, no investigation has yet examined whether pomegranate and beetroot juices can modulate recovery biomarkers within a real-life training program for amateur soccer players.

Therefore, the aim of this study was to investigate the efficacy of pomegranate and beetroot juices supplementation in facilitating recovery among Algerian amateur soccer players. This will be assessed through the decrease of biomarkers associated with muscle damage and inflammation (namely CK and CRP) alongside their normal weekly training program, which incorporates daily training sessions and competitive matches.

2 Subjects and Methods

2.1 Participants

A cohort of eighteen healthy amateur male soccer players was recruited for this investigation (mean ± SD: age, 24.5 ± 5.2 years; height, 180.0 ± 0.5 cm; body mass: 76.1 ± 14.2 kg). The exclusion criteria were as follows: (1) allergy to pomegranate, beetroot, or any of its derivatives; (2) the presence of a serious clinical pathology or significant medical antecedents; and (3) the use of any anti-inflammatory, analgesic, or antioxidant medication in the month preceding the initiation of the experiment trial.

For the study's duration, participants were instructed to maintain their habitual dietary patterns while abstaining from the consumption of any anti-inflammatory or antioxidant supplements. Furthermore, they were directed not to initiate any new exercise training regimens. The flowchart detailing participant allocation is described in Figure 1.

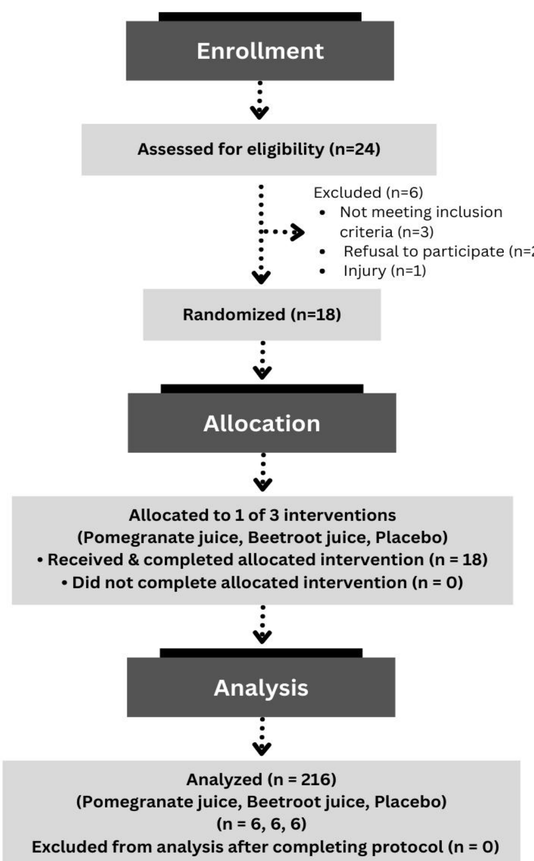

Figure 1. Flow Diagram of the Experimentation Study and Participant Allocation According to the Intake of Beetroot Juice, Pomegranate Juice, or Placebo Drink

2.2 Experimental Design and Procedures

This study was conducted in strict accordance with the Declaration of Helsinki. Prior to enrolment, written informed consent was obtained from all participants after receiving detailed information about the study procedures, potential risks and benefits, and their right to withdraw at any time without penalty. Participants were additionally assured that their data would be utilized solely for research purposes and that confidentiality would be rigorously maintained.

Formal permission to conduct the research was granted by an amateur football club competing in the Algerian Regional Secondary Division Football League, allowing experimentation during the training sessions of the team's 2021–2022 season. The experimentation period extends from November 2021 to January 2022.

The investigation employed a randomized, placebo-controlled design. Participants were instructed to adhere to their customary daily routines, training schedules, and nutritional diets to determine the effects of juice supplementation within the context of their regular football training and lifestyle. All players participated in the same training sessions throughout the experiment period. To standardize the weekly training load across the cohort, players who did not participate in matches or who only played in half-time matches were invited to take part in a short friendly match or an additional training session prescribed by the coach.

Participants were randomly assigned using a computer-generated randomization sequence with concealed allocation. Block randomization (block size of 6) was employed to ensure balanced group sizes. The allocation sequence was concealed through sequentially numbered, opaque, sealed envelopes prepared by an independent researcher not involved in recruitment or assessment. All participants, investigators, and outcome assessors remained blinded to group assignment throughout the study period.

The placebo beverage (colored water) was meticulously matched to the active interventions in terms of visual appearance (color), consistency, and packaging. Food-grade coloring was incorporated into water to mimic the appearance of the natural juices. This placebo was designed to preserve blinding integrity while providing no bioactive compounds. This approach aligns with standard randomized controlled trial protocols, where the placebo group serves as a control condition, enabling the assessment of the specific physiological effects attributable to the bioactive compounds in the pomegranate and beetroot juices, as distinct from any potential psychological or behavioral effects of participating in a supplementation protocol.

2.3 Training protocol

The experimentation period comprised seven microcycles, with each microcycle defined as a 7-day period training featuring a standardized distribution of training loads. Players engaged in their habitual football workout routine which consisted of 4 to 5 sessions per microcycle with a uniform structure and intensity distribution. This periodization model reflects the standard weekly training structure in competitive soccer, allowing for systematic training load management and recovery planning.

One high-intensity training (HIT) session was systematically scheduled per microcycle, with timing adjusted based on competitive match frequency. The HIT sessions served as the standardized exercise stimulus for evaluating muscle damage and inflammatory responses. Each training session lasted approximately 120 minutes and followed a consistent structure: 15–20-minute warm-up, followed by soccer-specific technical and tactical exercises including small-sided games, high-intensity running drills, tactical positioning exercises, and physical conditioning components (sprint training, speed endurance, strength, and power development). This training structure represents typical amateur-level soccer preparation and ensured consistent exercise stimulus across all participants.

We selected the HIT session to evaluate the impact of the juices on muscle damage and inflammation markers. The HIT session included: Warm-up; Reduced games without goal gradient (6 vs 6); High-intensity intervals (5 seconds of high-intensity activity repeated 3 times with recovery periods); Medium-intensity intervals (15 seconds of activity repeated 4 times for close matches); Long-intensity intervals (30 seconds repeated 4 times for spaced matches); Plyometric jumps + speed training (20 m sprints); Goal shooting practice; Recovery periods: 5 seconds between spaced matches, 15 seconds between close matches. Figure 2 displays the training microcycles and the integrated study design.

2.4 Preparation of Pomegranate Juice (POJ), Beetroot Juice (BEJ), and Placebo Beverage

The natural juices were extracted from beetroot (*Beta vulgaris*) and pomegranates (*Punica granatum*) purchased from a local market employing a Democlip electric juicer. The resulting products were 100% pure natural juices. Participants in the placebo (PLA) group received water adulterated with food-grade artificial coloring (E120) to visually match the visual appearance of the natural juices ensuring blinding without providing bioactive compounds.

All beverages were prepared and distributed to participants on the same day. Aliquots of each juice type were frozen at -20°C for phytochemical analysis. The total flavonoid content was quantified using the aluminum

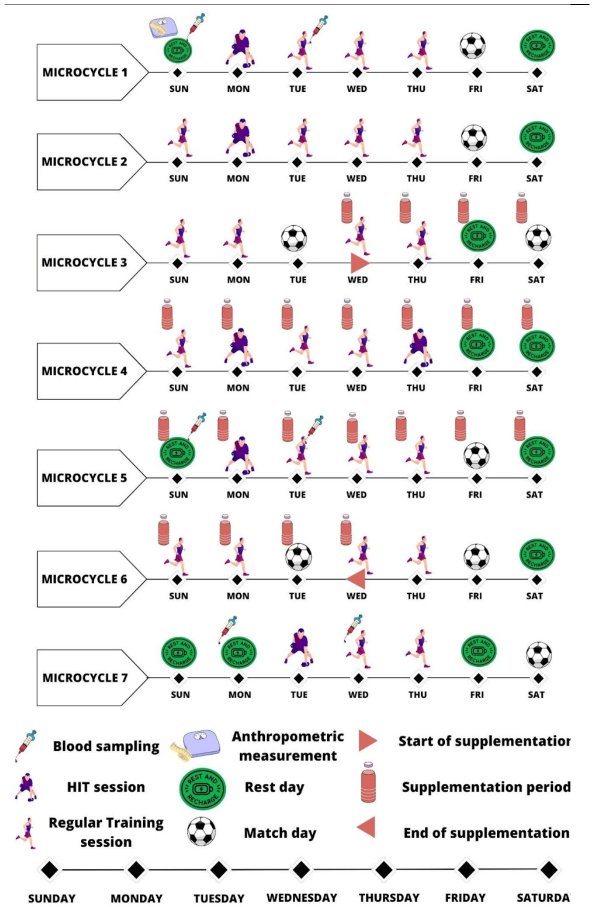

Figure 2. Schematic Representation of Training Microcycles and Study Design

trichloride method and was expressed as mg of quercetin equivalents per 100 mL (mg QE/100 mL) (Guendouze-Bouchefa *et al.*, 2015). The total phenolic content was determined with the Folin-Ciocalteu assay, with results expressed as milligrams of gallic acid equivalents per 100 mL (mg GAE/100 mL) (Haddadi-Guemghar *et al.*, 2014). Betalain content (Khatabi *et al.*, 2016) and total anthocyanin concentration (Boussaa *et al.*, 2020) were also spectrophotometrically determined.

Methodology for Juice and Placebo Supplementation

Each bottle of POJ contained 300 mL of undiluted juice, while a portion of BEJ contained 150 mL of concentrated beetroot juice. The 150 mL volume of BEJ was selected based on palatability considerations related to the characteristic earthy taste of beetroot. Preliminary taste tests indicated that larger volumes were perceived as unpalatable, which risked compromising participant. Consequently, this dosage represented the maximum acceptable volume to ensure 100% adherence while preserving the integrity of the blinding protocol.

The daily supplementation protocol was standardized over the 22-day intervention period as follows: The POJ group consumed 300 mL of juice twice daily (total: 600 mL/day), with one dose administered post-training (within 30 minutes) and the second dose 12 hours later. The BEJ group followed an identical schedule, consuming 150 mL of beetroot juice twice daily (total 300 mL/day). The PLA group consumed an equivalent volume of placebo beverage (150 mL twice daily) maintaining identical timing and blinding procedures. On non-training days, the initial dose was consumed in the afternoon simultaneously corresponding to the typical post-training time, with the second dose administered 12 hours later. Participants were provided with pre-measured individual servings and instructed to consume the entire portion at each administration. Compliance was monitored through daily self-report logs and the collection of empty containers, achieving 100% adherence throughout the study.

2.4 Sample Collection and Preparation

Venous blood samples were collected to analyze specific biological markers. In this study, creatinine (Crea), blood glucose (Gly), CK, and CRP were measured. Creatinine and glycemia were measured only at baseline to assess the participants' general health status.

A certified nurse collected 10 mL blood samples from participants on the training field, adhering strictly to safety and hygiene protocols. The samples were subsequently transported to a laboratory for analysis. To minimize analytical variations, all assays were performed in duplicate, and all samples were processed in a single laboratory by the same technician.

Blood samples were collected at six time points: 24 hours prior and further the first HIT session, before the supplementation period (Pre-supplementation); 24 hours before and following the HIT session, which corresponds to the 12th day of the supplementation period (T1), and finally 24 hours before and after the HIT session on day 22 of supplementation (T2). Participants were instructed to abstain from strenuous physical activity for at least 36 hours before each testing procedure. This duration aligns with established protocols in exercise physiology research, representing the minimum period required to achieve stable baseline measurements while remaining practically feasible for amateur athletes. This standardization was crucial for isolating the effects of our interventions from residual exercise-induced physiological perturbations. Participant height and body weight were also measured at baseline.

2.5 Sample Analysis

Creatine kinase (CK) activity was measured using an automatic biochemical analyzer (Mindray BS-330E, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) and a commercial reagent kit (CK-NAC IFCC, Biolabo SA Reagents). The established reference range for this assay in males aged 19 and over is 20–200 IU/L.

Serum CRP concentration was determined employing a CRP latex agglutination test (Biosystems CRP Kit, Spain) according to the manufacturer's instructions on an automated analyzer (Mindray BS-330E). The laboratory's normal reference value for this assay is reported at 6 mg/dL.

Basal plasma glucose levels were quantified using an automated analyzer and a commercially available kit (BioSystems). Creatinine concentration was measured using commercially kits (Spin React, Spain).

2.6 Statistical Analysis

Data were analyzed using XLSTAT software (version 2024.1.0, Addinsoft, Paris, France) and are expressed as mean ± standard deviation (SD). A one-way Analysis of Variance (ANOVA) was utilized to assess between-group differences between groups in participants' general characteristics and biochemical parameters at baseline. Within-group changes in CK and CRP levels (pre- vs. post-HIT) at each time point were evaluated using paired t-tests. The effects of supplementation type (POJ, BEJ, PLA) and time (pre-supplementation, T1, T2) on CK and CRP levels were analyzed using two-way repeated measures ANOVA (RM-ANOVA). When significant main effects or interactions were identified, post hoc comparisons were conducted using Tukey's Honest Significant Difference (HSD) test. Statistical significance was set at $p < 0.05$ for all analyses.

3 Results

3.1 Characteristics of the Study Participants

The baseline anthropometric characteristics and biochemical markers of the participants are displayed in Table 1. As indicated, these parameters demonstrated homogeneity across the three experimental groups. No significant differences were observed among the POJ, BEJ, and PLA groups for age (24.5 ± 7.0, 24.67 ± 4.23, and 25.5 ± 5.01 years, respectively), body mass, height, or BMI (all $p > 0.05$).

Table 1. Participants' Anthropometric and Biochemical Characteristics before the intervention period

Characteristics	POJ group (n = 06)	BEJ group (n = 06)	Placebo group (n = 06)
Anthropometric Characteristics			
Age (Years)	24.5 ± 7.0[a]	24.67 ± 4.23[a]	25.5 ± 5.01[a]
Weight (kg)	78.97 ±11.93[a]	73.08 ± 9.64[a]	76.38 ± 10.90[a]
Height (cm)	178.00 ± 0.5[a]	178.00 ± 0.5[a]	183.00 ± 0.5[a]
Body mass index (kg/m^2)	24.79 ± 3.24[a]	22.93 ± 2.35[a]	22.58 ± 5.07[a]
Biochemical Characteristics			
Baseline CK (IU/L)	134.80 ± 19.92[a]	125.60 ± 25.30[a]	139.50±20.22[a]
Baseline CRP (mg/mL)	0.50 ± 0.43[a]	0.57 ± 0.39[a]	0.50 ± 0.52[a]
Glycemia (g/L)	0.83 ± 0.10[a]	0.86 ± 0.05[a]	0.91 ± 0.08[a]
Creatinine (mg/L)	9.41 ± 0.88[a]	9.39 ± 0.95[a]	9.72 ± 1.47[a]

POJ: pomegranate juice; BEJ: beetroot juice; PLA: placebo; BMI: body mass index; CK: creatine kinase; CRP: C-reactive protein. Data are expressed as mean ± Standard Deviation.
Identical superscript letters indicate non-significant differences between groups (p > 0.05) and different letters represent significant differences

Similarly, baseline biochemical markers including CK, CRP, glycemia, and creatinine exhibited no significant inter-group differences. This confirms the success of randomization procedure and establishes appropriate baseline equivalence among the groups (Table 1).

3.2 Phytochemical Composition of the Tested Juices and Placebo

The phytochemical analysis revealed distinct yet complementary bioactive profiles for the two juices (Table 2). POJ demonstrated a higher total polyphenol content (417.71 vs 395.02 mg/100mL) and contained anthocyanins (0.14 mg/100 mL) absent in BEJ, while BEJ was uniquely rich in betalains (107.02 mg/100 mL). These compositional differences may underlie the comparable, yet mechanistically distinct, physiological effects observed in the present study.

Table 2. Phytochemical composition of the tested juices and placebo

Characteristics	POJ (n = 3)	BEJ (n = 3)	PLA (n = 3)
Total phenols (mg GAE/100 mL)	417.71 ± 0.42[a]	395.02 ± 0.04[b]	ND
Flavonoids (mg QE/100 mL)	126.14 ± 2.75[a]	114.38 ± 2.26[b]	ND
Betalains (mg /100 mL)	ND	107.02 ± 1.96	ND
Anthocyanin (mg cyanidin-3-glucoside/100 mL)	0.14 ± 0.007	ND	ND

POJ: pomegranate juice; BEJ: beetroot juice; PLA: placebo; ND: not detected; GAE: gallic acid equivalents; QE: quercetin equivalents. Results are expressed as mean ± standard deviation of three replicates (n = 3). Different letters represent significant differences (p < 0.05). Different superscript letters indicate significant differences between samples (POJ, BEJ and PLA), while identical letters indicate no significant differences.

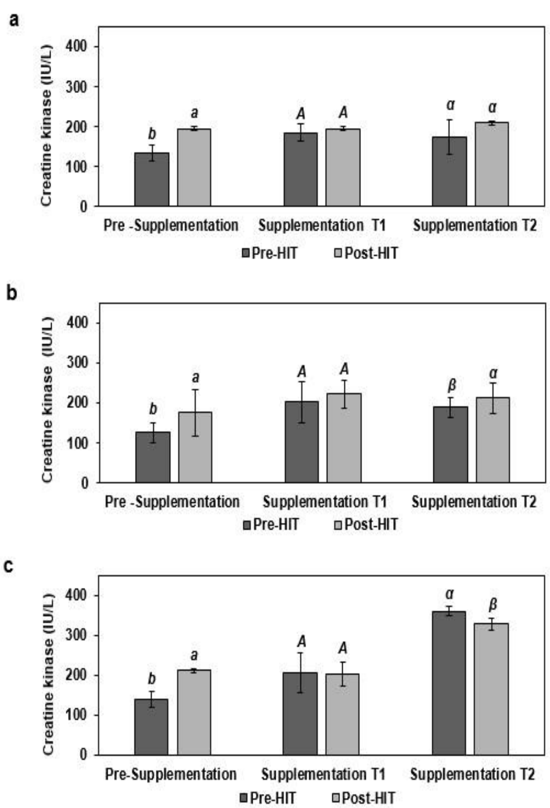

Figure 3. Effect of Sampling Times (pre-HIT; post-HIT) and Supplementation Types (A: POJ; B: BEJ; C: PLA) on Creatine Kinase Activity in Amateur Soccer Players (pre-supplementation; supplementation T1; supplementation T2)

3.3 Effect of Exercise on CK Activity

Figure 3 illustrates the average CK activity before and after the high-intensity training session across different supplementation times (pre-supplementation, supplementation T1, and supplementation T2) for each intervention group (POJ, BEJ, and PLA).

Initially, all groups exhibited a significant increase in CK activity following the HIT session, with values rising from 134.8 IU/L to 194.4 IU/L in the POJ group, from 125.6 IU/L to 175.92 IU/L in BEJ, and from 139.5 IU/L to 211.17 IU/L in the placebo group (p = 0.000, p = 0.031, and p < 0.0001, respectively), indicating measurable muscle damage. In T1, no group showed a significant difference between pre- and post-HIT values. In T2, BEJ showed a significant increase in CK levels (from 188.27 to 211.9 IU/L, p = 0.047), PLA showed a slight decrease in CK activity (from 359 to 327.75 IU/L, p = 0.001), while POJ demonstrated no significant difference (174.29 IU/L versus 209.3 IU/L, p = 0.053).

3.4 Effect of Supplementation on CK Activity

The effects of POJ, BEJ, and PLA on CK activity in amateur soccer players, measured pre- and post-HIT across all supplementation periods, are presented in Figure 4.

Pre-HIT condition (Figure. 4a): A repeated-measures ANOVA (RM-ANOVA) revealed a significant effect of the supplementation type (F (2, 33) = 39.10; p < 0.0001), the supplementation time (F (2, 66) = 111.26; p < 0.0001), and a significant interaction between these factors (F (4, 66) = 40.33; p < 0.0001) on pre-HIT CK activity. Significant differences were also observed between the PLA and POJ (p < 0.0001) and BEJ (p = 0.000). There was no significant difference between POJ and BEJ (p = 0.847). Pre-HIT CK activity changed significantly throughout the study, with significant differences observed between all measurement points.

Prior to supplementation, CK activity was comparable among the three groups (POJ: 134.80 ± 19.92 IU/L; BEJ: 125.60 ± 25.30 IU/L; PLA: 139.50 ± 20.22 IU/L; p > 0.05). At supplementation T1, the values remained similar (POJ: 184.50 ± 21.41 IU/L; BEJ: 201.88 ± 50.89 IU/L; PLA: 204.33 ± 49.53 IU/L; p > 0.05). Post-hoc analysis revealed

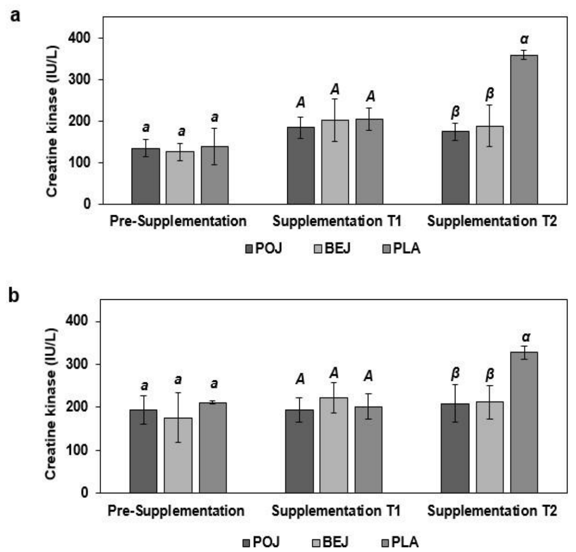

Figure 4. Comparison of the effect of different types of supplementations (POJ, BEJ, and PLA) on creatine kinase activity in amateur soccer players at different sampling times (A: Pre-HIT; B: Post-HIT) according to the duration of supplementation (pre-supplementation; supplementation T1 supplementation T2)

Means with different letters among groups (POJ, BEJ, and PLA)) within the same supplementation period are significantly different (p < 0.05). Means with the same letters indicate no significant difference (p > 0.05). Values are expressed as mean ± SD (n = 6 per group). The pre-supplementation analysis represents baseline responses before any intervention, while T1 and T2 represent responses at 12 and 22 days of supplementation, respectively.

that at supplementation T2, the PLA group (359.00 ± 11.39IU/L) had significantly higher CK activity than POJ (174.29 ± 43.95 IU/L) ($p < 0.001$) and BEJ (188.27 ± 26.29 IU/L) ($p < 0.001$).

Within-group comparisons revealed that in BEJ group, CK activity increased significantly from pre-supplementation (125.60 ± 25.30 IU/L) to T1 (201.88 ± 50.89 IU/L) ($p <$ 0.002), with no significant change between T1 and T2 ($p >$ 0.05). In POJ, no significant variation was observed between pre-supplementation and T1 ($p > 0.05$), nor between T1 and T2 ($p > 0.05$). The comparison between pre-supplementation and T2 confirmed that CK activity was significantly elevated in the PLA group (from 139.50 ± 20.22 IU/L to 359.00 ± 11.39 IU/L; $p < 0.0001$) and BEJ (from 125.60 ± 25.30 to 188.27 ± 26.29 IU/L; $p = 0.02$), but not in POJ ($p > 0.05$). However, at T2, CK values remained significantly higher in the PLA group than in the two supplemented groups ($p < 0.001$).

Post-HIT Condition (Figure 4b): The RM-ANOVA revealed significant main effects for supplementation type (F (2, 33) = 17.268; $p < 0.0001$), supplementation time (F (2, 66) = 27.603; $p < 0.0001$), as well as a significant interaction between supplementation type and time (F (4, 66) = 15.870; $p < 0.0001$). The POJ and BEJ groups showed a significant difference from PLA ($p = 0.006$), and (p = 0.012), respectively. Moreover, CK activity exhibited significant changes throughout the study, with notable differences identified between pre-supplementation and supplementation T2 ($p =$ 0.000) and between supplementation T1 and T2 ($p = 0.002$).

At all three measurement points, the average CK levels in the PLA group (pre-supplementation: 211.17 ± 3.99 IU/L; T1: 202.00 ± 30.35 IU/L; T2: 327.75 ± 15.32 IU/L) were significantly higher than those in POJ (pre-supplementation: 194.40 ± 32.93 IU/L; T1: 193.72 ± 29.23IU/L; T2: 209.30 ± 44.25 IU/L) and BEJ (pre-supplementation: 175.92 ± 57.90 IU/L; T1: 221.63 ± 35.45IU/L; T2: 211.90 ± 38/.7 IU/L) ($p < 0.0001$ for all comparisons). No significant differences were observed between groups POJ and BEJ at any time point ($p >$ 0.05).

Within the PLA group, CK activity increased significantly from pre-supplementation to T1 and then to T2 ($p < 0.05$), while no significant variation was observed in POJ and BEJ between the three measurement times ($p > 0.05$).

3.5 Effect of Exercise on C-Reactive Protein

The average CRP levels before and after HIT at different supplementation time points (pre-supplementation, T1, and T2), depending on the type of supplementation (POJ, BEJ, and PLA), for each group are displayed in Figure 5.

In the POJ group (Figure 5a), a significant increase was observed in the pre-supplementation time, with values rising from 0.5 mg/mL ± 0.43 to 0.75 mg/mL ± 0.37 ($p = 0.039$). In contrast, no significant differences were observed between pre- and post-HIT values at supplementation T1 and T2 for any of the groups (POJ, BEJ, and PLA) ($p>0.05$). The BEJ and PLA exhibited no significant variation throughout the experimentation period ($p = 0.106$ and $p = 0.166$), respectively, with their pre- and post-HIT values remaining stable.

3.6 Effect of Supplementation on CRP

Figure 6 illustrates the effects of POJ, BEJ, and PLA supplementation on CRP concentration, measured pre- and post-HIT across all supplementation periods.

In contrast to the findings for CK activity, the type of juice supplementation exhibited no significant effect on CRP levels, either before or after an HIT session ($p > 0.05$). However, a significant main effect of the supplementation period was observed in the pre-HIT condition (F (2, 66) = 3.700; $p = 0.030$) and post-HIT (F (2, 66) = 8.672; $p <$ 0.001). Despite this overall effect revealed by the RM-

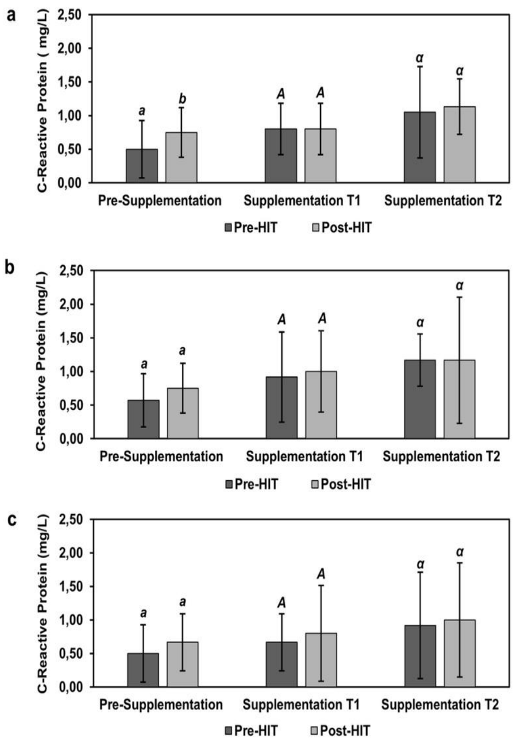

Figure 5. Effect of sampling times (pre-HIT; post-HIT) and supplementation types (A: POJ; B: BEJ; C: PLA) on C-reactive protein concentration in amateur soccer players according to time of supplementation (pre-supplementation; T1 supplementation; T2 supplementation)

Means with different letters between sampling times (pre-HIT vs. post-HIT) within the same supplementation period are significantly different (p < 0.05). Means with the same letters indicate no significant difference (p > 0.05). Values are expressed as mean ± SD (n = 6 per group). The pre-supplementation analysis represents baseline responses before any intervention, while T1 and T2 represent responses at 12 and 22 days of supplementation, respectively.

ANOVA analysis, post hoc analyses showed no significant differences between the different measurement points (pre-supplementation, T1, T2). On average, pre-HIT CRP levels were 0.50 ± 0.43 mg/L for the POJ group, 0.57 ± 0.39 mg/L for the BEJ group, and 0.50 ± 0.52 mg/L for the PLA group before supplementation, and remained at 0.71 ± 0.36, 0.50 ± 0.52, and 0.42 ± 0.44 mg/L at T2, respectively, with no significant difference ($p > 0.05$). An identical pattern was observed in the post-HIT condition, with mean values remaining stable and comparable between groups throughout the supplementation period ($p > 0.05$).

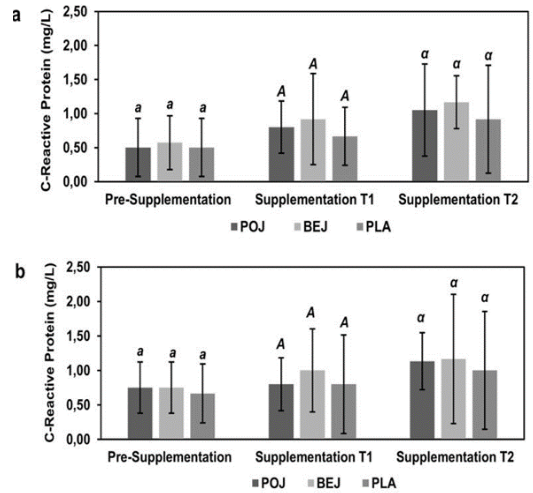

Figure 6. Comparison of the effect of the different types of supplementations (POJ, BEJ, and PLA) on C-reactive protein concentration in amateur soccer players at different sampling times (A: Pre-HIT; B: Post-HIT) according to the duration of supplementation (pre-supplementation; supplementation T1 supplementation T2)

Means with different letters among groups (POJ, BEJ, and PLA)) within the same supplementation period are significantly different (p < 0.05). Means with the same letters indicate no significant difference (p > 0.05). Values are expressed as mean ± SD (n = 6 per group). The pre-supplementation analysis represents baseline responses before any intervention, while T1 and T2 represent responses at 12 and 22 days of supplementation, respectively.

4 DISCUSSION

This comparative investigation evaluated the effects of a 22-day supplementation regimen with pomegranate juice (600 mL/day) and beetroot juice (300 mL/day) on markers of muscle damage and inflammation in amateur soccer players during regular training. The findings present nuanced results, which, while limited by a constrained sample size, offer valuable insights. The participant cohorts were relatively homogeneous, as indicated by pre-intervention anthropometric and biochemical data, confirming that all groups were well-matched and entered the study under comparable physiological conditions, free from pathological concerns. It is pertinent to note that this study was conducted in the immediate post-COVID-19 pandemic period. compliance was exemplary, with 100% adherence to the consumption of the assigned beverages. Furthermore, no adverse effects, such as bloating, nausea, vomiting, or diarrhea, were reported throughout the intervention.

4.1 Phytochemical Composition of the Tested Juices

The pomegranate juice utilized in this study exhibited a total phenolic compound (TPC) content of 417.71 ± 10.42 mg GAE/100 mL, which corresponds to the values reported for aril juices in the literature. For instance, Mahdavi *et al.*, (2010) measured a TPC of 421.4 ± 0.5 mg GAE/100 mL. While Nilova *et al.*, (2023) reported 411.2 ± 8.2 mg GAE/100 mL. Conversely, Zaouay *et al.* (2012) observed a lower range of 133.93 to 350.06 mg of EAG/100 mL across different cultivars (Zaouay *et al.*, 2012). These discrepancies are likely attributable to variations in analytical methodologies, extraction and pasteurization procedures, and differences between varieties. (Mahdavi *et al.*, 2010). The flavonoid content of aril juice reaches 126.14 ± 2.75 mg EQ/100 mL, which falls within the range observed for juices obtained from different cultivars, with reported values between 20.8 to 189.4 mg QE/100 mL (Fernandes *et al.*, 2017).

A total anthocyanin concentration in our sample was 0.14 ± 0.007 mg cyanidin-3-glucoside per 100 mL. This is considerably lower than the range of 16.8 to 132.8 mg/100 mL reported by Sepúlveda *et al.* (2010), though it aligns more closely with the 0.18 to 16.85 mg/100 mL found in certain Iranian varieties (Akhavan *et al.*, 2015). The low anthocyanin concentrations in our juice may be explained by freezer storage as anthocyanins are known for their sensitivity to environmental factors and processing leading to structural degradation over time (Xue *et al.*, 2024).

Regarding the beetroot juice, the measured TPC was 395.02 ± 0.04 mg GAE/100 mL, which is significantly lower than the value of 2250 mg GAE/100 mL reported by Bazaria & Kumar, (2016) and higher than the 160.69 mg GAE/100 mL estimated by Clifford *et al.*, (2017). The flavonoid content in our sample also differed from the 45.25 ± 2.14 mg/100 mL reported by Mlček *et al.* (2016) for fresh juice. Such variability is likely influenced by geographical, climatic, and agricultural origins, as well as factors including cultivar type, maturity stage, and genetics (Deseva *et al.*, 2020; Rababah *et al.*, 2023). The total betalain content measured in our raw juice reached 107.02 ± 1.96 mg/100 mL, close to the values observed by Kruszewski *et al.* (2023) with 100.1 ± 3.4 mg/100 mL, including 75.3 ± 2.8 mg/100 mL of betacyanins and 24.8 ± 0.6 mg/100 mL of betaxanthins (Kruszewski *et al.*, 2023). Nevertheless, these values remain notably lower than the high concentration of 236.51 mg/100 mL observed by Tumbas Šaponjac *et al.*, 2020.

4.2 Effect of Exercise on CK Activity

The observed post-HIT elevation in CK activity before the supplementation remains consistent with numerous studies, following eccentric exercise protocol (Philpott *et al.*, 2018) and after a soccer match (Souglis *et al.*, 2015). Exercise-induced muscle damage, particularly from eccentric contractions, is a well-documented cause of increased serum CK levels, which can persist for up to 72 hours after intense exercise (Ispirlidis *et al.*, 2008). The significant increase in CK measurements in the present study confirms that the HIT session successfully induced typical myocyte damage.

4.3 Effect of the Supplementation on CK Activity

The supplementation with both pomegranate and beetroot juices demonstrated significant efficacy in attenuating the increase in CK activity compared to the placebo, although levels did not return to pre-experiment baseline. This decrease was observed both before and after training sessions, suggesting a potential beneficial impact on CK activity under both resting and post-exercise conditions.

Numerous studies have investigated the effects of POJ on CK, with promising, albeit sometimes inconsistent. For instance, Ammar *et al.* (2016) reported that POJ supplementation in weightlifters accelerated CK recovery kinetics, suggesting that a high-polyphenol concentration (2.56 g/500 mL) could be a practical and effective intervention for stabilizing muscle damage (Ammar *et al.* 2016). This aligns with our findings, as the polyphenol concentration of our juice was 2.51 g per 600 mL of pomegranate juice. However, in our study, the effect of pomegranate juice was only evident after 22 days of supplementation, indicating that efficacy may depend on supplementation duration, exercise type, and the specific composition of the bioactive compounds.

Concerning beetroot juice, our results align with those of Sinaga *et al.*, (2021b) who reported a significant reduction in serum CK levels following supplementation. Similarly, Montenegro *et al.*, (2017) observed an attenuated CK increase in triathletes supplemented with a betalain-rich concentrate. In contrast, Clifford *et al.*, (2016b) found that although exercise induced a significant increase in plasma CK levels, neither a high dose (250 mL) nor a reduced dose (125 mL) of beet juice significantly altered this response compared to placebo (Clifford *et al.*, 2016b). Clifford *et al.*, 2017 in a second study observed that supplementation with 500 mL of beetroot juice for four days after muscle-damage-inducing exercise had no significant effect on serum CK level in team sports players (Clifford, Berntzen, *et al.*, 2016). Furthermore, Daab *et al.*, 2021 found that beetroot juice supplementation (300 mL for 7 days) did not affect plasma creatine kinase compared with placebo in footballers after performing the Loughborough Intermittent Shuttle Test (Daab *et al.*, 2021).

This observed reduction in CK is likely attributable to the antioxidant and anti-inflammatory properties of the juices' bioactive compounds. The high nitrate (NO3-) content of beetroot juice is a source of interest (Milton-Laskibar *et al.*, 2021). Additionally, beetroots contain several other compounds, including phenolic acids, flavonoids, and betalains (Wootton-Beard *et al.*, 2014). Its betalain content also possesses potent antioxidant and anti-inflammatory properties (Desseva *et al.*, 2020; Milton-Laskibar *et al.*, 2021). These compounds could influence exercise-related physiological responses independently of nitrates (Lorenzo Calvo *et al.*, 2020). Pomegranate juice has demonstrated considerable free radical scavenging capacity compared to other beverages, including orange, grapefruit, grape, and cranberry (AlMatar *et al.*, 2019). This activity is due to the existence of antioxidant polyphenols such as ellagitannins and anthocyanins (Matthaiou *et al.*, 2014). In aril juice, anthocyanins constitute the principal group of phenolic compounds with antioxidant and anti-inflammatory functions. Anthocyanins can reduce CK, inflammation, and lipid peroxidation for muscle function recovery and increase maximal oxygen consumption and antioxidant capacity (Khairani *et al.*, 2020). Additionally, this juice is a rich source of ellagitannins, a type of polyphenol that also possesses antioxidant and anti-inflammatory properties (Harty *et al.*, 2019).

The observed reduction in creatine kinase (CK) levels following pomegranate juice supplementation can be attributed to several biochemical mechanisms. Anthocyanins, the major phenolic compounds in pomegranate juice, contribute through:

- Direct antioxidant activity by scavenging reactive oxygen species (ROS), thus protecting muscle cell membranes from oxidative damage (Ammar *et al.*, 2017).
- Upregulation of endogenous antioxidant enzymes such as superoxide dismutase and catalase via redox-sensitive pathways (Machin *et al.*, 2014).
- Improvements in lipid profiles and attenuation of exercise-induced oxidative stress, as demonstrated in placebo-controlled trials (Ammar *et al.*, 2020).
- And suppression of inflammatory signaling, including reduced expression of nuclear factor-κB (NF-κB), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), as shown in metabolic models using pomegranate peel extract (Rak-Pasikowska *et al.*, 2024).

Similarly, beetroot juice provides complementary effects via its high nitrate and betalain contents, enhancing nitric oxide bioavailability, vascular function, and oxygen delivery to tissues. These benefits, combined with antioxidant and anti-inflammatory properties, contribute to reduced muscle damage and accelerated recovery (Clifford *et al.*, 2015; Haider & Folland, 2014; Jones *et al.*, 2018).

Although the effect of supplementing both juices only became apparent after 22 days, the difference in dosage between pomegranate and beetroot juices (600 mL vs. 300 mL) is particularly interesting. Despite this disparity, the effects observed remained comparable, raising questions about the underlying mechanisms of action. POJ, with its greater volume, contains a higher concentration of bioactive compounds, notably anthocyanins (0.14 ± 0.007 mg cyanidin-3-glucoside/ 100 mL^{-1}), absent in BEJ. Furthermore, beet juice is recognized for its richness in betalains (107.02 ± 1.96 mg 100 mL^{-1}), pigments with equally powerful antioxidant properties, absent in pomegranate juice. This difference in composition could explain why, despite its smaller volume, beet juice produces effects comparable to those of pomegranate juice. Betalains and anthocyanins, secondary plant metabolites, possess similar chemical properties, biological functions, and color spectra. However, they never coexist together in plants (Harty *et al.*, 2019). This agrees with the results of the phytochemical analysis of the studied juices. Given the numerous bioactive properties of these two compounds separately, it would be interesting to test their combined effects on athletes' recovery.

While our results demonstrated no significant difference between the effects of POJ and BEJ on CK levels, this may be due to the limited statistical power of the current investigation, and further research with larger sample sizes is warranted to support these findings.

4.4 Effect of Exercise on C-Reactive Protein

C-Reactive Protein (CRP) is an acute-phase reactant synthesized by the liver in response to inflammation, infection, or tissue lysis. In sports medicine, it serves as a valuable marker for monitoring an athlete's inflammatory status and recovery (Mehmood *et al.*, n.d.). Exercise-induced muscle damage typically induces a rise in inflammatory markers including CRP, which can persist for several days post-exertion (Junior *et al.*, 2023).

Contrary to this expectation, our study did not report significant changes in CRP levels, despite the concurrent elevations in CK. This discrepancy may be explained by the magnitude of muscle damage. Indeed, according to the results of Isaacs *et al.*, 2019 CRP was higher in individuals with high CK reactivity (CK activity exceeding 1000 U/L) compared to those with low reactivity (CK activity less than 1000 U/L) (Isaacs *et al.*, 2019). The creatine kinase values observed in our study fall within the CK activity range of people with low reactivity. According to Beals *et al.*, 2017 it is just as likely that an eccentric protocol could not induce a systemic inflammatory response and that the initial muscle damage and myofibril disruption were insufficient to provoke a systemic inflammatory response (Beals *et al.*, 2017). Furthermore, baseline CRP levels in young, lean, healthy males are typically below 2 mg/L (Beavers *et al.*, 2010), which corresponds to the values obtained throughout the current study.

4.5 Effect of Supplementation on C-Reactive Protein (CRP)

In contrast to the CK results, our study showed no significant effect of juices on CRP levels, either before or after intense training ($p > 0.05$). This observation is consistent with the results of a meta-analysis by Wang *et al.*, (2020), which concluded that pomegranate supplementation does not significantly affect CRP levels, either overall or specifically in men. Similarly, Daab *et al.*, (2021) found that CRP levels in footballers were unaffected by beet juice supplementation (300 mL for 7 days) compared with the placebo group after performing the Loughborough Intermittent Shuttle Test. In contrast to this work and the results of our study, another study reported that beetroot juice supplementation significantly reduced CRP levels in athletes after submaximal exercise compared to the control group (18.00 ± 5.65 mg/L VS 74.66 ± 23.85 mg/L ($p = 0.000$)) (Sinaga *et al.*, 2021a).

Given that the exercise protocol did not induce a pronounced inflammatory state (CRP < 6 mg/L), it is difficult to establish whether the anti-inflammatory properties of the juices were simply not engaged. The absence of a significant inflammatory stimulus precludes a definitive conclusion regarding their efficacy in countering exercise-associated inflammation.

The inconsistencies between our results and those of other studies could be due to significant variations in research methods. A meta-analysis by Rickards *et al.* (2021) highlighted considerable variability in outcomes related to polyphenol-rich foods, and recovery from exercise-induced muscle damage (EIMD). While they found slight improvements in muscle soreness and strength recovery, they also reported considerable inconsistencies especially in biochemical markers such as creatine kinase (CK) and C-reactive protein (CRP). These inconsistencies are likely tied to the wide range of polyphenol sources (such as cherry, pomegranate, blueberry, and blackcurrant), varying dosages, different supplement forms (whole food, juice, and extract), and differing lengths of interventional —all of which make it challenging to compare and combine study results. The diverse participant groups (trained vs. untrained), various types of exercise (eccentric vs. endurance), and inconsistent outcome measures also made it difficult to pin down overall effects accurately.

Our study contributes to this discourse by employing a controlled, real-world training setup to compare two distinct, bioactive-rich juices. We tested two different juices that are rich in separate bioactive compounds, beetroot juice and anthocyanins in pomegranate juice—to increase the real-life relevance of our findings. In line with some of the studies reviewed by Rickards *et al.* (2021) we observed steady CRP levels throughout the supplementation periods. This might be because the training program didn't cause a strong inflammatory response, or because our participants, who were already trained, had low baseline inflammation. These results highlight the need for context-specific research that uses consistent exercise protocols and biomarker assessments to better understand when and how polyphenol-rich supplements might help with recovery from EIMD

Limitations

This study is not without limitations. The primary constraint is the small sample size (n=18, six per group), which may limit the statistical power and generalizability of the findings. Furthermore, the supplementation period was limited to 22 days due to logistical constraints within the soccer team's schedule, a duration which may have been suboptimal for inducing maximal physiological adaptations. The absence of detailed phytochemical analyses of the juice preparations precludes precise correlation between specific bioactive constituents and observed effects.

5 Conclusions

This study demonstrates that 22-day supplementation with either pomegranate juice (600 mL/day) or beetroot juice (300 mL/day) yields comparable efficacy in attenuating biochemical markers of muscle damage and supporting recovery following soccer-specific training. These findings support the therapeutic potential of bioactive compounds such as anthocyanins and betalains in modulating oxidative stress and inflammatory pathways involved in muscle recovery. Given the controlled, yet highly sport-specific, nature of this intervention, and the inherent complexity of exercise–nutrition interactions, the extrapolation of these findings to other athletic populations or training modalities warrants caution. Future research is therefore required, focusing on larger and more heterogeneous athletic cohorts, standardized exercise protocols, and thorough phytochemical profiling of supplements to fully elucidate dose–response relationships and the underlying mechanistic pathways.

Despite these constraints, the results offer practical insights for athletes and coaches. Regular supplementation with either pomegranate or beetroot juice into nutritional strategies could form part of an integrative recovery protocol, for intermittent, high-intensity sports such as soccer, to potentially reduce muscle damage and expedite recovery without adverse effects. Future research should focus on more diverse cohorts and include comprehensive compositional profiling of functional beverages to identify the most efficacious compounds and optimal dosing regimens. Furthermore, individual tolerance, dietary context, and potential interactions with other recovery modalities should be considered when integrating such beverages into sports nutrition programs to enhance athletic training and competition outcomes.

Ethics approval and consent to participate

All procedures involving human participants adhered to the ethical standards of the institutional and/or national research committee and to the Declaration of Helsinki and its subsequent amendments or comparable ethical standards.

Ethical approval for this research was granted by the research team of the Functional and Sports Nutrition Sciences at the Institute of Physical Education and Sports, Laboratory of Physical Activity Sciences (LSTAPS, code C1680300), Scientific Council, University Brahim Soltane Chaibout, under the ethical approval code CE- N001/2021. Informed consent was obtained from all individual participants included in the study. Formal approval was also granted by the technical staff of the participating football club.

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