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Evaluation of Pomegranate Seed Powder Based Drink Premixes for Athletes

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Abstract

Pomegranate (Punica granatum) is a poly phenolic-rich fruit with a strong in vitro and in vivo antioxidant potential, which has been promising in topics related to the endurance capacity, reduction of muscle damage, and exercise-induced oxidative stress. The purpose of the study was to determine the efficiency of a pomegranate-based pre-workout nutritional intervention as an instrument of improvement in exercise performance. This study aimed at assessing the effect of a pre-workout pomegranate drink on exercise performance. pomegranate seed powder was prepared and analyzed for nutritional composition and antioxidant properties. The sensory evaluation was conducted to assess the acceptability and preference of the pre-workout drink among athletes. The pomegranate seed powder was carefully prepared to make sure it is consistent every time. Its nutritional content and antioxidant activity was checked using standard methods that was ranged between 0.4-5.4% moisture, 1.5-11.5% ash, 0.4-4.5% protein, 0.1-4.5% fiber, 5-56.7% fat and 9.4-12% NFE,

respectively. The overall acceptability of different treatments ranged between 5.5-8.1%. The TPC content of different juices ranged between 19.3-26 mgGAE/100g with12.5-45% antioxidant activity. The data was collected and analyzed to determine the effectiveness of the drinkas a pre-workout supplement.

Introduction

Nutrition support is through strategic use of dietary supplementation and food-based interventions, linking with exercise to enhance performance and recovery (Benardot, 2024). The point at which nutrition and exercise intersect has received considerable attention recently with regard to the effects of specific nutritional interventions on athletic performance and recovery (Mozaffarian et al., 2024). In a relevant pursuit of optimal exercise outcomes and enhanced recovery, the role of targeted nutrition strategies toward these aims has recently come into focus. Among the panoply of nutritional interventions put forward, pomegranate-based drinks seem to influence exercise performance and recovery because of their distinct phytochemical profile (Bonilla et al., 2021). Pomegranate is one of the fruits extremely rich in poly phenolic compounds. The fruit contains punicalagins, anthocyanin, and ellagic acid--first-class antioxidants and anti-inflammatory agents (Saparbekova et al., 2023). These bioactive compounds were observed to have various health benefits associated with improved cardiovascular functionality and reduced oxidative stress. Some recent studies have reported that pomegranate juice consumption exerts positive effects on exercise performance, increasing endurance, reducing muscle soreness, and reducing oxidative damage occurring during exercise. The main mechanisms by which these effects can be achieved include improvements in nitric oxide bioavailability, increased blood flow, and higher mitochondrial function (Túñez et al., 2020). Pomegranate-based drinks are consumed before workout in order to enhance endurance and reduce oxidative damages, while protein is taken after workouts in order to enhance the synthesis of muscle protein and speed up recovery. Thereby, nutrition support provided in this work will be more tailored to the needs of athletes/fitness enthusiasts living in Pakistan by using the specific properties of pomegranate for the optimization of physical outcomes (Panara et al., 2023). These compounds also play critical roles in the neutralization of free radicals, mitigation of oxidative stress, and alleviation in inflammation, hence making pomegranate a beneficial dietary ingredient with respect to exercise performance and recovery. In the same breath, the presence of these bioactive phytochemical substances not only helps to aid heart health but also has implications for better endurance and faster recovery via increased blood flow and less soreness of the muscles. This phytochemical diversity underscores the potential of pomegranate to be an effective nutritional intervention in athletic performance and recovery, particularly in the context of pre-workout nutrition (Journey, 2024). In Pakistan, the pomegranate fruit, known as ‘ anar,” holds cultural significance and is widely consumed in various forms, including juice. The increasing awareness of health and fitness has led to a surge in the popularity of pomegranate juice among fitness enthusiasts. Local markets are seeing a rise in the availability of fresh pomegranates and ready-to-drink pomegranate juices, often marketed as health supplements. Moreover, the agricultural sector in Pakistan has the potential to benefit economically from the cultivation of pomegranates, given their rising demand both domestically and internationally. This not only supports local farmers but also aligns with the global trend of seeking natural, health- promoting beverages. Pomegranate juice is emerging as a potent ally in workout nutrition, offering numerous benefits that enhance exercise performance and recovery. Its rich nutritional profile and antioxidant properties make it an excellent choice for athletes and fitness enthusiasts in Pakistan. As awareness of its health benefits continues to grow, pomegranate juice may play a significant role in the dietary practices of those engaged in physical fitness,

contributing to both individual health and local agricultural economics (Figueiredo et al., 2020). Pre-workout nutrition is important to set the body up for exercise. The goals would be a sufficient amount of energy substrates and nutrients promoting high performance and counteracting early fatigue. Addition of pomegranate seed powder into a pre-exercise drink was hypothesized to promote exercise performance since it would add a nutrient-dense concentration of antioxidants and complex carbohydrates, which may help in sustaining higher energy levels and temper exercise-induced oxidative stress. Other ingredients, such as chia seeds also bring home the effect of energy availability on general physical activity and sustaining performance. Pomegranate drink as pre-workout for endurance and reducing muscle fatigue. The nutrition support strategy for this study will be oriented to the requirements of athletes in Pakistan, keeping in minds the local dietary habits and ingredient availability therein (Khan et al., 2022).

MATERIAL AND METHODOLOGY

The present study was carried out at the Department of Human Nutrition and Dietetics Riphah International Faisalabad, Pakistan. As nutritional interventions enhanced training performance, the research aimed to determine the efficiency of a pomegranate seed powder pre-workout drink. Raw material was purchased from local market of Faisalabad.

Fresh pomegranates will be washed, deseeded, and the arils dried at 45-50°C using a food dehydrator. The dried arils will be ground into a fine powder using an electronic blender and sieved through a 250-micron screen to ensure uniformity. The powder will then be stored in airtight plastic bags, labeled and kept in a cool, dry place to maintain freshnessand prevent contamination (Khademi et al., 2024).

Treatment Plan

Treatments	Pomegranate powder (%)	Chia seeds (%)	Stevia (%)
T_1	10%	45%	45%
T_2	20%	40%	40%
T_3	30%	35%	35%
T_4	40%	30%	30%

The pomegranate seed powder will undergo proximate analysis to determine its nutritional composition, Moisture Content, Ash Content, Crude Protein, Crude Fat, CrudeFiber: and NFT according to the AOAC (2024) method.

The total phenolic content of juices was determined according to the Folin-Ciocalteu method. 0.4 mL sample was added to a 10 mL volumetric flask, having 3.6 mL of distilled water. Folin-Ciocalteu reagent (0.4 mL) was added to the mixture. After 5 min, 4 mL of 7% sodium carbonate was added. The absorbance was measured at 765 nm using a UV-visual spectrophotometer. The result was expressed as gallus equivalent (mg GAE /100 mL) (Ghalegi Ghalenoe et al., 2021).

0.1 mM of methanolic DPPH stem solution was generated using 10 mg DPPH in 125 mL of methanol in a 250 mL volumetric flask. A 0.4 mL diluted sample was added to a test tube containing 5.6 mL of methanolic DPPH. Test tubes sealed with parafilm were incubated in a water bath at 37°C for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The results were expressed as μmol TE/100 mL. (Wern, Haron & Keng. 2016).

Sensory evaluation was conducted to assess the acceptability and preference of the pre-workout drink. This evaluation helps in understanding the drink's overall acceptability (W, 2024)

The data was analyzed to find the average value of each attribute and acceptability. The results were used to identify any significant differences in the sensory attributes of the drink (Montgomery et al., 2007).

RESULTS AND DISCUSSION:

Sports nutrition includes proper dietary approaches for athletes, injuries and wound recovery during performance and prevention of malabsorption and diseases affecting supports performance. The athletic sports trend worldwide is increasing due to its positive impact on health, endurance, and quality of life. Fruits like pomegranate, grown in tropical and subtropical regions, possess a major number of antioxidants, as well as minerals such as copper, phosphorus, selenium, manganese, magnesium, calcium, iron, and zinc, with 5% protein and fiber content each. It is beneficial for weight maintenance, reduced risk of CVD and neurological disorder. It provides insist energy as a drink to the body with internal wound healing and reduced oxidative stress.

Compositional Analysis of pomegranate seeds powder and chia seed powder

Parameters	Pomegranate seed powder	Chia seeds powder
Moisture	0.46+0.9	5.44+0.6
Ash	1.87+0.1	11.4+0.9
Crude Protein	4.8+4.45	0.4+12.4
Crude fiber	4.84+8.84	0.11+00.5
Crude Fat	5.3+0.5	56.76+11.4
NFE	12.18+0.4	9.4+6.63

Based on the analysis, the moisture content of pomegranate seed was recorded as 0.46+0.9 to 5.44+0.6 across different samples. The relatively high moisture content observed in pomegranate seed may be attributed to their strong water absorption capacity, which benefits seed germination and early growth. Hollenberg et al. (2019)reported a value of 7.15±0.3%, supporting the nutritional characteristics identified in the current study. Aishwarya and Anisha, (2014) recorded a moisture content of 3.1±0.058%.

The ash content determined in pomegranate seeds was recorded as1.87+0.1, 11.4+0.9 across the analyzed samples. These values suggest that pomegranate seeds generally possess a substantial mineral content, as indicated by their relatively high ash percentages. The current findings regarding the influence of processing on the proximate composition of pomegranate powder seeds align closely with the work of Adesina, (2019) who reported ash content values ranging from 4.61% to 5.36%.

The analysis revealed that the protein content of pomegranate seed powder varied across samples, with values recorded at 4.8+4.45, 0.4, +12.4 respectively. The findings of this study are comparable to those reported by Adesina, (2019), who found a protein content of 21.2±0.6%. In their assessment of the physicochemical and antioxidant properties of pomegranate powder seeds**.**

The analysis recorded the crude fiber content of pomegranate seed powder at 4.84+8.84, 0.11+00.5 across different samples. Comparable findings have been reported in earlier research. For instance, Aishwarya & Anisha, (2014) reported a much lower fiber content of 0.98±0.053%, while Arif et al. (2013)

identified a crude fiber value of 5.91±0.08% in their nutritional assessment of pomegranate powder seeds. These varying results highlight the influence of factors such as seed variety, cultivation practices, and processing methods on the fiber content of pomegranate seeds.

The analysis revealed that the crude fat content of pomegranate seeds varied considerably among samples, with recorded values of 5.3+0.5 to 56.76+11.4. These results confirm that pomegranate seed powder are an excellent source of beneficial fats. Comparable findings have been reported by several researchers. Zhang et al. (2020) documented a fat content of 24.5±0.4% while examining the physicochemical properties and antioxidant activity of pomegranate seeds.

The NFE percentages for the pomegranate seed samples were 12.18+0.4, 9.4+6.63. These findings suggest that pomegranate seeds, especially certain samples, contain a substantial amount of carbohydrates and other nitrogen-free compounds. The notably high NFE values indicate the presence of digestible carbohydrates and soluble nutrients, contributing to the overall energy content of pomegranate powder seeds.

Effects of Storage and Treatment on TPC (mg GAE/100g) of Pomegranate seed powder drink

Treatments	Storage (Days)			
	0	15	30	Means
T1	19.45±0.11	19.39±0.12	19.31±0.14	19.38±0.12[b]
T2	274.40±40	261.10±39	246.70±40	260.73±39.60[a]
T3	22.35±0.19	22.33±0.15	22.28±0.18	22.332±0.17[b]
T4	23.32±0.17	23.30±0.15	23.26±0.18	23.29±0.16[b]
Means	84.09±19.20[a]	81.53±18.32[ab]	77.88±19.33[b]	

The analysis of variance (mean squares) for total phenolic content (TPC) indicated that the treatment had a significant impact on the TPC of both pomegranate seed powder drink. A gradual decline in TPC was observed in all drink samples throughout the storage period. The recorded TPC values were 19.45 ± 0.11, 19.31 ± 0.14, 274.40 ± 40, 246.70 ± 40, 22.35 ± 0.19, 22.28 ± 0.18, 23.32 ± 0.17, and 23.26 ± 0.18 mg GAE/100g, respectively the total phenolic content (TPC) in pomegranate powder seed drink treatments (T1, T2, T3, and T4) remained relatively consistent throughout the storage period, showing only minor reductions by the 30th day. Ahmad et al. (2019) investigated the development of low-calorie ready-to-serve (RTS) pomegranate powder seed drink using varying concentrations of stevia. Their findings revealed an increase in the total phenolic content of beverages prepared with optimal amounts of stevia, ranging from T1 to T4 (19.45 ± 0.11 to 23.26 ± 0.18 mg GAE/100g). However, a significant decline in the antioxidant activity of these treatments was observed over the storage period.

Effects of Storage and Treatment on DPPH (%) of Pomegranate seed powder drink

Treatments	Storage (Days)			
	0	15	30	Means

T1	13.81±0.21	13.57±0.22	13.38±0.24	12.587±0.22[c]
T2	56.61±6.32	44.40±6.45	37.20±6.22	45.07±6.33[b]
T3	15.85±0.26	15.69±0.15	14.99±0.22	14.51±0.21[c]
T4	16.83±0.18	16.74±0.19	16.59±0.22	15.72±0.19[c]
Means	40.03±3.50[a]	33.56±3.46[b]	27.84±3.35[c]	

The mean square values for DPPH indicated that both treatment and storage duration had a highly significant effect on the antioxidant activity (DPPH) of pomegranate seed powder drink. A reduction in DPPH values was observed pomegranate seed powder drink over the storage period. The DPPH values for treatments T1, T2, T3, and T4 declined from 16.59 ± 0.22 to 13.81 ± 0.21, 13.38 ± 0.24, 56.61 ± 6.32, 37.20 ± 6.22, 15.85 ± 0.26, 14.99 ± 0.22, and 16.83 ± 0.18, respectively. Among the pomegranate seed drink treatments, T3 showed the highest antioxidant activity, whereas in the case of pomegranate powder seed drink, the highest antioxidant activity was recorded in T4. The decrease in DPPH values observed in pomegranate drink during the 30-day storage period may be attributed to the antioxidant activity of bioactive compounds naturally present in the drink. Płudowski et al. (2023) investigated the osmotic effects of pomegranate combined with various drink concentrations and observed a decline in DPPH radical scavenging activity over the storage period, with values decreasing from 24.4–34.2% to 19.4–33.7%

Effects of Storage and Treatment on Flavor of Pomegranate seed powder drink

Treatments	Storage (Days)			
	0	15	30	Means
T1	8.16±0.03	8.08±0.04	7.99±0.02	8.07±0.09[c]
T2	8.57±0.03	8.49±0.02	8.42±0.02	8.49±0.07[a]
T3	7.95±0.03	7.89±0.02	7.82±0.02	7.88±0.07[d]
T4	7.78±0.03	7.74±0.03	7.68±0.04	7.73±0.10[f]
Means	8.11±0.25[a]	7.05±0.22[b]	7.97±0.21[c]	

The analysis of mean square values for sensory properties indicated that both the different treatments and storage duration had a highly significant impact on sensory characteristics, including flavor. The mean comparison demonstrated the impact of both storage duration and different treatments on the flavor of pomegranate seed drinks. Treatments T1 to T4 varied based on the type and concentration of stevia, a natural sweetener, incorporated into the formulations. At the beginning of storage (day 0), the control sample for pomegranate seed (T2) recorded the highest mean flavor score of 8.13±0.03, followed by the control sample for pomegranate drink (T1), which had a mean score of 8.26±0.03. As storage progressed, a gradual decline in flavor scores was observed across all treatments, including the controls. By the end of the 30-day storage period, the lowest flavor scores were noted in T3 (8.01±0.03) and T4 (7.84±0.03). Among the samples, T2 consistently maintained higher flavor scores compared to the other treatments, indicating its suitability as the control for pomegranate drink. On the other hand, treatments T2, T3, and T4, which incorporated higher levels of stevia, experienced a more pronounced reduction in flavor over time.

Srinivas, (2015) also documented comparable outcomes, where attributes such as flavor demonstrated significant variations influenced by formulation and storage and sensory quality of fruit-based beverages and dairy products exhibited a decreasing trend during storage but remained acceptable.

Effects of Storage and Treatment on Color of Pomegranate seed powder drink

Treatments	Storage (Days)			
	0	15	30	Means
T1	8.26±0.03	8.17±0.04	8.04±0.02	8.15±0.09[a]
T2	8.13±0.03	8.04±0.02	7.95±0.02	8.04±0.07[b]
T3	8.01±0.03	7.94±0.02	7.88±0.02	7.94±0.07[c]
T4	7.84±0.03	7.79±0.03	7.72±0.04	7.78±0.10[d]
Means	8.06±0.25[a]	7.98±0.22[b]	7.89±0.21[c]	

The analysis of mean square values for sensory properties indicated that both the different treatments and storage duration had a highly significant impact on sensory characteristics, including color. Throughout the storage period, changes in the color of the functional beverages were noted, although these changes were less pronounced in the treated samples compared to the control. The control beverages tended to develop a darker color primarily due to the hydrolysis of sucrose into fructose and glucose, which subsequently participated in the Maillard reaction. In contrast, when sucrose was replaced with stevia, the beverages exhibited a lighter color, since sucrose essential for the formation of typical color through browning reactions was absent. The mean comparisons for sensory attributes such as color revealed that while the sensory scores declined significantly over a storage period of 30 days, the products remained within acceptable limits as evaluated by the sensory panel. Srinivas, (2015) documented comparable outcomes, where color demonstrated significant variations influenced by formulation and storage and sensory quality of fruit-based beverages and dairy products exhibited a decreasing trend during storage but remained acceptable.

Effects of Storage and Treatment on Overall Acceptability of Pomegranate seed powder drink

Treatments	Storage (Days)			
	0	15	30	Means
T1	8.25±0.03	8.17±0.04	8.09±0.02	8.17±0.09[a]
T2	8.12±0.03	8.05±0.02	7.96±0.02	8.04±0.07[b]
T3	8.02±0.03	7.93±0.02	7.87±0.02	7.94±0.07[c]
T4	7.83±0.03	7.78±0.03	7.69±0.04	5.82±0.10[d]
Means	8.05±0.25[a]	7.98±0.22[b]	7.90±0.21[c]	

At the start of the storage period (day 0), the control treatments (T1 and T2) prepared with pomegranate and chia seed powder drink recorded relatively high scores for overall acceptability. According to the mean comparison, treatment T1 achieved the highest mean score of 8.26±0.03, followed by T2 with 8.13±0.03, T3 with 8.01±0.03, and T4 with 7.84±0.03. The statistical indicators confirmed that these differences were significant differences were observed among the treatments, with T1 showing a

statistically significant variation compared to T2, T3, and T4. As the storage period extended to 30 days, a slight reduction in overall acceptability was noted for both control treatments. In the case of treatments containing stevia, it was observed that overall acceptability generally declined with increasing stevia concentration and longer storage duration. Specifically, treatments T1 through T4, which incorporated different levels of stevia, consistently received lower acceptability scores than the control samples at all stages of storage. This indicates that higher amounts of stevia may have an adverse effect on the overall acceptability of the drink products. Furthermore, a steady decrease in acceptability scores was recorded across all treatments over time. For example, by the 30th day of storage, treatment T1 recorded a mean overall acceptability score of 7.93±0.07, which was higher than the scores for treatments T2, T3, and T4. Similar findings were reported by Silva, Silva et al. (2021) who evaluated the physicochemical, antioxidant, and sensory characteristics of guava pulp juice and noted results consistent with those observed in this study. Likewise, Srinivas, (2015) also documented comparable outcomes, where attributes such as color, flavor, and taste demonstrated significant variations influenced by formulation and storage sweetness and overall sensory quality of fruit-based beverages and dairy products exhibited a decreasing trend during storage but remained acceptable.

CONCLUSION:

Athletes' nutrition deals with the health issues, injuries and nutrient deficiencies in athletes, managing mental stress, preventing bone loss and injuries.Pomegranate is a natural supplement that fulfill body with instant energy, maintains body mineral and protein requirement. The antioxidant activity of pomegranate juice helps in releasing stress, combat free radical, prevent chronic disease and malabsorption of nutrients. Regular consumption of pomegranate juice may recover body's nutritional requirement and energy level of athletes. Pomegranate seed can also be used as supplements or energy drink for instant energy or dealing with micronutrient deficiencies in general population.

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