

Formulation of nitrate-rich gummies from Arugula leaves for sports personnel

Keerthana M¹, Dhanya H M², Ganavi³

^{1,2,3} Padmashree Institute of Management and Sciences, Bangalore

Abstract—The development of nitrate-rich gummies for enhancing sports performance represents an innovative approach to optimizing exercise capacity and recovery. Nitrate, primarily derived from natural sources such as beetroot and arugula, has been shown to improve nitric oxide (NO) production, which can enhance vasodilation, reduce oxygen consumption, and improve athletic performance. This study focuses on formulating nitrate-rich gummies specifically designed to support sports performance and recovery. Gummies incorporating concentrated nitrate extracts from arugula were developed to create a convenient, effective, and appealing supplement for athletes. Key aspects of formulation included optimizing nitrate concentration, ensuring product stability, and achieving a desirable taste and texture. Stability tests were conducted to monitor nitrate retention and ensure the gummies maintained efficacy throughout their shelf life. The study focuses on the formulation and analysis of arugula-based gummies enriched with jaggery, ginger, lemon, and agar-agar. A total of 200 mL of gummy syrup was prepared, yielding 14 gummies, with additional samples prepared for proximate analysis. Nutritional analysis showed the gummies contained 98.11 Kcal, 2.5g protein, 0.82g fat, 20.17g carbohydrates, 1.67g fibre, 0.861g ash, and 73.96% moisture. Nitrate content was determined to be 5952.99mg, and microbial analysis, conducted after 2 months of shelf life, identified the presence of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus epidermidis* across different media.

Index Terms—Nitrate-rich gummies, Sports performance, Nitric oxide (NO), Arugula extract, Exercise capacity, Endurance, Recovery, Sports nutrition,

I.INTRODUCTION

The *Eruca vesicaria* plant, also known as arugula, rucola, roquette, and rocket, has gained popularity in many media outlets for its potential health benefits. It

is commonly found in supermarkets as a leafy green vegetable and has recently become available as an extract. Rocket species (*Eruca* spp. and *Diplotaxis* spp.) are becoming increasingly important leafy salad crops across the world. Numerous scientific research papers have been published in recent years on the potential health benefits of phytochemicals in leaves, such as flavonoids and isothiocyanates. Other research of note has also examined the unique taste and flavour properties of leaves, which can be hot, peppery, bitter, and sweet depending on the genotype and phytochemical composition. While research into these aspects is increasing, some studies lack cohesion and depth of knowledge of commercial breeding and cultivation practices, making the interpretation and application of results difficult. Both forms are touted for their high nitrate content, which could play a significant role in arugula's use as a dietary therapeutic. In a semi-randomised controlled trial, Jonvik *et al.* confirmed that vegetables rich in nitrate (e.g., arugula, beetroot, and spinach) increased plasma nitrate and nitrite concentrations and subsequently decreased blood pressure in healthy adults. Moreover, Jonvik *et al.* have concluded that nitrate-rich vegetables (e.g., arugula, beetroot, and spinach), consumed in various forms, may serve as acute dietary nitrate supplements. Current literature suggests that diets high in nitrate content tend to improve cardiovascular health by lowering blood pressure and preventing declines in endothelial function (e.g., flow-mediated dilation [FMD], pulse wave velocity [PWV], and/or shear rate [SR]) after an ischemic stimulus, as measured by brachial artery FMD. Of all cruciferous vegetables, arugula has one of the highest known nitrate contents, yet there is limited research on its effectiveness in treating chronic diseases and promoting endothelial function. Athletes, both elite

and recreational, are interested in using legal means to improve their performance. While consuming a balanced diet is important for maintaining general health and for fueling and recovering from training, many athletes also use dietary supplements to enhance performance during competition. Examples of these supplements include caffeine, creatine, sodium bicarbonate, beta-alanine, and, most recently, inorganic nitrate. The evidence base for the efficacy of nutritional “ergogenic aids” is notoriously mixed, and nitrate is no exception. However, recent meta-analyses do support the suggestion that dietary nitrate, which is normally ingested by athletes in the form of 12 beetroot juices, has the potential to enhance performance in several types of sport and exercise activities. Moreover, unlike other supplements that are mainly of value to athletes, nitrate supplementation may also provide cardiovascular health benefits to the general (nonathletic) public via its well-described reduction of resting blood pressure, and it may benefit older or diseased populations by enhancing the efficiency of locomotion, muscle O₂ delivery, and/or muscle power. Inorganic nitrate is a natural component of many foods, notably green leafy vegetables, but is also used as a preservative in products such as cured meats. For many years, despite relatively weak evidence, nitrate (NO₃⁻) and nitrite (NO₂⁻) were classified as carcinogens, but more recent evidence indicates that nitrate may be a key bioactive component in salad vegetables, whose consumption is encouraged to promote human health. This has prompted a re-evaluation of the risks and benefits of dietary nitrate. Arugula's popularity is attributed to both its taste and health benefits. Like other plant-based foods, it is associated with a lower risk of obesity, diabetes, heart disease, and all-cause mortality. Arugula is rich in cancer-fighting compounds, particularly sulforaphane, which is found in cruciferous vegetables. This compound has shown promise in inhibiting cancer cell growth and is being investigated for its potential in treating various cancers, including melanoma and prostate cancer (Kim and Park, 2016). Sulforaphane is believed to work by inhibiting histone deacetylase (HDAC) enzymes, which are involved in cancer progression. Arugula also contains alpha-lipoic acid, an antioxidant that can lower patients' blood glucose levels, improve insulin sensitivity, and prevent

oxidative stress in diabetic patients. Additionally, it has been shown to reduce nerve damage in people with diabetes (Shay *et al.*, 2009). This leafy green is high in nitrate, which has been associated with lower blood pressure, reduced oxygen needs during exercise, and improved athletic performance. Arugula provides about 64 mg of calcium per two cups, contributing to bone health, muscle function, and blood clotting. It also contains 369 mg of potassium, which supports muscle function and can help mitigate the effects of sodium, benefiting those with high blood pressure (Lidder and Webb, 2013). Furthermore, arugula is rich in folate, a B vitamin important for DNA production and crucial for pregnant women to prevent neural tube defects. It also offers vitamin C, an antioxidant that supports immune function and enhances iron absorption. Overall, arugula is a nutrient-dense food, low in sugar, calories, carbohydrates, and fat, making it a valuable addition to a healthy diet (Kumari Shubha, November 2019).

II. MATERIALS AND METHODS

Arugula Leaves (*Eruca sativa*) are leafy greens with a peppery flavour, often used in salads and as a garnish. It is rich in vitamins A, C, and K, as well as minerals like calcium, potassium, and nitrate. Fresh arugula leaves were sourced from an e-commerce site. Approximately 100 grams were used per experiment or serving. Lemon (*Citrus limon*) is a citrus fruit known for its high vitamin C content and acidic properties. It is widely used for its flavour and health benefits. Fresh lemons were obtained from a local farmer's market. One whole lemon was used for each application to extract juice and zest. Jaggery is a traditional, unrefined sweetener made from sugarcane or palm sap. It contains minerals like iron, magnesium, and potassium, which are often lost in refined sugars. Sourced from a local grocery, 20 grams of jaggery were used per experiment to provide a natural sweetener and nutritional benefits. Ginger (*Zingiber officinale*) is a root widely used for its spicy flavour and medicinal properties, including anti-inflammatory and antioxidant effects. Fresh ginger was acquired from a local supermarket, with 10 grams used per preparation to enhance flavour and as a widely used food preservative that inhibits the growth of Mould and yeast. It is commonly used in

small quantities to maintain freshness and extend the shelf life of food products. Purchased from a certified food-grade chemical supplier from Amazon, potassium sorbate was used at a concentration of 0.1% to ensure safety while preventing spoilage.

Arugula Leaves were washed thoroughly with water and air-dried. They were then cut into smaller pieces and ground in a mixer for use in the final product. The lemon was washed, and the juice was extracted. One whole lemon gave 20ml of juice. Jaggery was finely grated to ensure even distribution in the mixture, then melted and boiled until caramel-like in consistency. The ginger root was peeled, ground, and then macerated to extract its juice. This juice was then added directly to the mixture. The preservative was dissolved in distilled water and added to the mixture at the final stage to prevent microbial growth.

A mixture was created by combining arugula leaf juice, lemon juice, melted jaggery, and ginger juice. This mixture was thoroughly stirred to ensure all ingredients were well-integrated. Potassium sorbate was then added to the mixture, which was stirred again and left to sit for 30 minutes to let the flavours blend. The final product was evaluated for taste, texture, and preservation properties.

III. RESULTS AND DISCUSSIONS

A total of 200 ml of the sample was prepared. Out of which, 14 gummies, each weighing 15g, were prepared.

Table 1: Nutritional analysis of the product

Parameter	Value (g)
Energy	98.11 $\pm$ 0
Crude protein	2.5 $\pm$ 0
Crude fat	0.82 $\pm$ 0.18
Total carbohydrate	20.17 $\pm$ 1.37
Crude fibre	1.67 $\pm$ 1.15
Ash	0.861 $\pm$ 0.03
Nitrate	5.95
Total moisture content	73.96 $\pm$ 0.48

*Nutritional value per 100 g of gummies; values are mean $\pm$ standard deviation for three replications.

IV. CONCLUSION

The prepared gummy sample (200 ml yielding 14 gummies, each weighing 15 g) was evaluated for proximate composition, nitrate content, and microbial safety. Proximate analysis showed an energy value of 98.11 kcal/100 g, with 20.17 g carbohydrates, 2.5 g crude protein, 0.82 g crude fat, 1.67 g crude fibre, and 73.96% moisture. A high nitrate concentration of 5952.99 mg was detected, indicating potential performance-enhancing properties but also necessitating further safety evaluation regarding acceptable intake levels.

Microbial analysis after 2 months of storage revealed contamination and spoilage. Identified microorganisms included *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus epidermidis*, detected on Nutrient Agar, Potato Dextrose Agar, MacConkey Agar, Eosin Methylene Blue agar, and Mannitol agar. These findings emphasise the importance of improved preservation methods and storage conditions to enhance the safety and shelf stability of nitrate-rich gummies.

The development of nitrate (NO_3^-) rich gummies represents a promising approach in sports nutrition, given the recognised ergogenic effects of dietary nitrate on muscle function and performance. Nitrate supplementation enhances muscle contractility, mitochondrial efficiency, blood flow, and neuromuscular function through the $\text{NO}_3^-/\text{NO}_2^-$ pathway, which enables nitric oxide (NO) production independent of oxygen supply and nitric oxide synthase (NOS). This mechanism is particularly beneficial during intense exercise, hypoxic conditions, ageing, and disease states in which oxygen availability is limited. Nitric oxide also regulates calcium (Ca^{2+}) activity, supports mitochondrial respiration, and improves oxygen and nutrient delivery to muscles, thereby enhancing endurance and physical performance. Nitrate-enriched gummies offer a convenient and palatable alternative to traditional supplements, with potential applications for athletes and individuals with reduced muscular performance.

REFERENCES

- [1] Aguilera, Y., Martín-Cabrejas, M. A., & González de Mejía, E. (2016). Phenolic compounds in fruits and beverages consumed as part of the Mediterranean diet: their role in prevention of chronic diseases. *Phytochemistry reviews*, 15, 405-423.
- [2] Allen, J. D. (2023). Nitric oxide as a mediator of exercise performance: NO pain, NO gain. *Nitric Oxide*, 136, 8-11.
- [3] Anese, M., Manzocco, L., Nicoli, M. C., & Lerici, C. R. (1999). Antioxidant properties of tomato juice as affected by heating. *Journal of the Science of Food and Agriculture*, 79(5), 750- 754.
- [4] Ashor, A. W., Lara, J., & Siervo, M. (2017). Medium-term effects of dietary nitrate supplementation on systolic and diastolic blood pressure in adults: a systematic review and meta-analysis. *Journal of hypertension*, 35(7), 1353-1359.
- [5] Bell, L., Oruna-Concha, M. J., & Wagstaff, C. (2015). Identification and quantification of glucosinolate and flavonol compounds in rocket salad (*Eruca sativa*, *Eruca vesicaria* and *Diplotaxis tenuifolia*) by LC-MS: Highlighting the potential for improving nutritional value of rocket crops. *Food Chemistry*, 172, 852-861.
- [6] Bondonno, C. P., Croft, K. D., Ward, N., Considine, M. J., & Hodgson, J. M. (2015). Dietary flavonoids and nitrate: effects on nitric oxide and vascular function. *Nutrition reviews*, 73(4), 216-235.
- [7] Bors, W., Heller, W., Michel, C., & Saran, M. (1990). [36] Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. In *Methods in Enzymology* (Vol. 186, pp. 343-355). Academic Press.
- [8] Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30.
- [9] Bryan, N. S., Ahmed, S., Lefer, D. J., Hord, N., & von Schwarz, E. R. (2023). Dietary nitrate biochemistry and physiology. An update on clinical benefits and mechanisms of action. *Nitric Oxide*, 132, 1-7.
- [10] Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of agricultural and food chemistry*, 49(6), 2774-2779.
- [11] Castro-López, C., Sánchez-Alejo, E. J., Saucedo-Pompa, S., Rojas, R., Aranda-Ruiz, J., & Martínez-Ávila, G. C. G. (2016). Fluctuations in phenolic content, ascorbic acid and total carotenoids and antioxidant activity of fruit beverages during storage. *Heliyon*, 2(9).
- [12] Chung, J. C., Chou, S. S., & Hwang, D. F. (2004). Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures. *Food Additives and Contaminants*, 21(4), 317-322.
- [13] Corleto, K. A., Singh, J., Jayaprakasha, G. K., & Patil, B. S. (2018). Storage stability of dietary nitrate and phenolic compounds in beetroot (*Beta vulgaris*) and arugula (*Eruca sativa*) juices. *Journal of Food Science*, 83(5), 1237-1248.
- [14] Doel, J. J., Benjamin, N., Hector, M. P., Rogers, M., & Allaker, R. P. (2005). Evaluation of bacterial nitrate reduction in the human oral cavity. *European Journal of Oral Sciences*, 113(1), 14-19.
- [15] Duarte, J., Francisco, V., & Pérez-Vizcaíno, F. (2014). Modulation of nitric oxide by flavonoids. *Food & function*, 5(8), 1653-1668.
- [16] Georgiev, V. G., Weber, J., Kneschke, E. M., Denev, P. N., Bley, T., & Pavlov, A. I. (2010). Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. *Plant foods for human nutrition*, 65, 105-111.
- [17] Gliszczynska-Świgło, A., Szymusiak, H., & Malinowska, P. (2006). Betanin, the main pigment of red beet: Molecular origin of its exceptionally high free radical-scavenging activity. *Food additives and contaminants*, 23(11), 1079-1087.
- [18] Gregoris, E., & Stevanato, R. (2010). Correlations between polyphenolic composition and antioxidant activity of Venetian propolis. *Food and Chemical Toxicology*, 48(1), 76-82.
- [19] Hendrickson, H. P., Kaufman, A. D., & Lunte, C. E. (1994). Electrochemistry of catechol-containing flavonoids. *Journal of Pharmaceutical and Biomedical Analysis*, 12(3), 325-334.
- [20] Jayaprakasha, G., & Patil, B. S. (2007). In vitro evaluation of the antioxidant activities in fruit

extracts from citron and blood orange. Food Chemistry, 101(1), 410–418.

- [21] Jayaprakasha, G. K., Girennavar, B., & Patil, B. S. (2008). Antioxidant capacity of pummelo and navel oranges: Extraction efficiency of solvents in sequence. LWT-Food Sci. Technology, 41(3), 376–384.
- [22] McQuillan, J. A., Dulson, D. K., Laursen, P. B., & Kilding, A. E. (2017). The effect of dietary nitrate supplementation on physiology and performance in trained cyclists. International journal of sports physiology and performance, 12(5), 684-689.
- [23] San Juan, A. F., Domínguez, R., Lago-Rodríguez, Á., Montoya, J. J., Tan, R., & Bailey, S. J. (2020). Effects of dietary nitrate supplementation on weightlifting exercise performance in healthy adults: A systematic review. Nutrients, 12(8), 2227.
- [24] Shannon, O. M., Duckworth, L., Barlow, M. J., Deighton, K., Matu, J., Williams, E. L., ... & O'Hara, J. P. (2017). Effects of dietary nitrate supplementation on physiological responses, cognitive function, and exercise performance at moderate and very-high simulated altitude—Frontiers in Physiology, 8, 401.
- [25] Thompson, C., Vanhatalo, A., Jell, H., Fulford, J., Carter, J., Nyman, L., ... & Jones, A. M. (2016). Dietary nitrate supplementation improves sprint and high-intensity intermittent running performance. Nitric Oxide, 61, 55-61.
- [26] Townsend, J. R., Hart, T. L., Haynes IV, J. T., Woods, C. A., Toy, A. M., Pihera, B. C., ... & Gonzalez, A. M. (2022). Influence of dietary nitrate supplementation on physical performance and body composition following offseason training in Division I athletes. Journal of Dietary Supplements, 19(4), 534-549.