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ENHANCED ARGININE YIELD FROM CO-EXTRACTED POMEGRANATE AND BROWN RICE AND ENCAPSULATION INTO POLYMERIC NANOPARTICLES

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ABSTRACT

This study aimed to enhance arginine yield through the co-extraction of pomegranate (Punica granatum) and brown rice (Oryza sativa), and to develop a polymeric nanoparticle system for its controlled delivery. Co-extraction was performed using aqueous blending and mild heating, followed by spray drying. Arginine content was quantified by HPLC after FMOC-Cl derivatization. Results showed that co-extraction produced significantly higher arginine levels than individual extracts. Arginine-rich extract was encapsulated using a sodium alginate-calcium chloride system via the coacervation method. The resulting nanoparticles showed a mean size of 598.67 nm and high encapsulation efficiency (88%). Stability studies over four weeks indicated that arginine-loaded nanoparticles maintained their physical integrity better than naked arginine powder, which showed discoloration and aggregation. This demonstrates that polymeric nanoparticles not only enhance arginine delivery potential but also improve storage stability. The findings suggest that combining natural plant matrices with nanotechnology is a promising strategy for amino acid-based therapeutic development.

KEYWORD: Pomegranate, Brown rice, Arginine, Natural product, Drug delivery system.

INTRODUCTION

In recent years, the use of natural products and plant-based materials for therapeutic and nutraceutical applications has gained increasing attention due to their biocompatibility, low toxicity, and multiple health-promoting effects. Among various bioactive compounds found in natural sources, amino acids play a pivotal role in supporting human health and metabolic functions. One such amino acid of particular interest is arginine, a semi-essential, conditionally indispensable amino acid that is widely recognized for its diverse physiological and pharmacological functions.[1-5]

Arginine serves as a precursor for the synthesis of nitric oxide (NO), a potent vasodilator that plays a crucial role in vascular homeostasis, immune modulation, neurotransmission, and wound healing. Additionally, arginine is involved in the urea cycle, which is essential for the detoxification of ammonia, and plays roles in protein synthesis, hormone secretion, and tissue repair. Due to these benefits, arginine supplementation has been investigated for a variety of clinical applications, including cardiovascular diseases, erectile dysfunction, immune enhancement, and metabolic disorders. Dietary sources of arginine include both animal and plant-based materials. However, there has been growing interest in identifying and optimizing plant-derived sources of arginine, especially for use in vegan, halal, and clean-label pharmaceutical formulations. Two natural candidates that show potential in this context are pomegranate (Punica granatum) and brown rice (Oryza sativa).[6-9] Both are rich in nutrients and bioactive compounds and are widely consumed in traditional diets for their health benefits.

Pomegranate is a fruit well known for its antioxidant, anti-inflammatory, and cardioprotective properties. It contains a wide variety of phytochemicals, including polyphenols (such as punicalagins, ellagic acid), anthocyanins, tannins, and organic acids. While its arginine content is not particularly high compared to leguminous sources, its enzymatic environment and acidic pH may support protein hydrolysis and amino acid liberation during extraction.[10] Brown rice, on the other hand, is a whole grain that retains the bran and germ layers, which are rich in fiber, vitamins, minerals, and proteins. Unlike white rice, brown rice contains higher levels of amino acids, including arginine. However, the bioaccessibility of these amino acids depends largely on the preparation and processing methods used. Traditional extraction methods, such as hot water extraction, enzymatic hydrolysis, or acidic treatment, are often employed to isolate amino acids from plant matrices. However, the yield and stability of amino acids can vary

depending on the source and method. Preliminary findings from our study indicated that co-extraction of pomegranate and brown rice under controlled conditions significantly enhanced the yield of arginine, compared to extracting each plant separately.[11-18] This suggests a possible synergistic effect between the two materials. It is hypothesized that the organic acids and polyphenols in pomegranate may facilitate protein denaturation or disrupt cell walls, enhancing the release of protein-bound amino acids from the brown rice matrix. This synergy could offer a natural, efficient strategy to boost the recovery of bioactive amino acids without the use of harsh chemicals.

Despite the nutritional value of arginine, its therapeutic application faces several limitations, including instability during processing and storage, poor bioavailability due to first-pass metabolism, and a short half-life in systemic circulation. To overcome these challenges, modern drug delivery systems have been developed, with nanotechnology-based platforms gaining prominence.[19-21] Polymeric nanoparticles offer a versatile and effective approach for encapsulating bioactive compounds, protecting them from degradation, controlling their release, and enhancing their absorption and therapeutic efficacy.

Polymeric nanoparticles are submicron-sized carriers formed from biodegradable and biocompatible polymers such as alginate, chitosan, or PLGA. These nanoparticles have been extensively studied for their ability to entrap hydrophilic or hydrophobic compounds, improve solubility, and provide targeted or sustained drug release. Among them, alginate-based nanoparticles crosslinked with calcium ions present a safe, economical, and environmentally friendly alternative. Alginate is a natural anionic polysaccharide obtained from brown seaweed, and it forms stable hydrogels in the presence of divalent cations such as calcium. These properties make it ideal for encapsulating polar molecules like arginine.[21-22]

In this study, we first aimed to enhance the yield of arginine through a novel co-extraction method using pomegranate and brown rice, followed by quantification using High-Performance Liquid Chromatography (HPLC) with pre-column derivatization for accurate detection. The arginine-rich extract was then encapsulated into polymeric nanoparticles prepared by the coacervation method using sodium alginate and calcium chloride. The prepared nanoparticles were characterized in terms of particle size, size distribution, polydispersity index (PDI), and encapsulation efficiency. Additionally, the stability of the nanoparticles was evaluated under accelerated storage conditions. By combining traditional food-based materials with modern drug delivery technology, this research proposes a dual innovation: improving the natural yield of a valuable bioactive compound and developing a delivery system to maximize its potential therapeutic effects. The study not only contributes to the field of natural product extraction and functional ingredient formulation but also aligns with the global movement toward sustainable, plant-based pharmaceutical solutions.[23-24]

To our knowledge, this is one of the first studies to explore the synergistic effect of co-extracting pomegranate and brown rice for enhanced arginine yield, and to subsequently encapsulate this extract using polymeric nanoparticles for controlled delivery applications.[25] The outcomes may offer insights for future development of nutraceutical products, functional foods, or novel pharmaceutical agents utilizing natural amino acid sources and nanotechnology.

METHODS

Reagents and equipment

Acetonitrile 99.9% HPLC Grade, QReC, Trifluoroacetic acid ≥ 99.9% HPLC Grade, Fisher Scientific, Citric Acid Anhydrous E330, RZBC (China), Potassium dihydrogen phosphate, Kemaus, Sodium hydroxide 98% (food grade), Vdells siam (Thailand).

Extraction process of brown rice and pomegranate

Firstly, fresh pomegranates were carefully selected, washed, and peeled to collect only the arils. The arils were then crushed using a blender to obtain the juice, which was filtered through a fine mesh to remove pulp and seeds, yielding pure pomegranate extract.

In parallel, brown rice was rinsed thoroughly to eliminate surface impurities and soaked in distilled water for several hours to soften the grains. After soaking, the rice was drained and blended with a measured volume of distilled water to create a slurry. This slurry was subjected to mild heating for about 30 minutes to facilitate the release of bioactive compounds.

On the mixture processes, 50 g of pomegranate extract and 25 g of brown rice extract were dissolved in 100 mL of deionized water. The solution was then spray dried at 70 C under continuously 500 rpm and flow rate as 12 ml/min. The finally, the powder was collected to further analysis.

Analysis total amount of Arginine amino acid

The analysis of arginine was performed using High-Performance Liquid Chromatography (HPLC) following a pre-column derivatization method. Initially, 10 mg/mL of extract the was prepared by hydrolyzing the protein material with 6 N hydrochloric acid (HCl) at 110°C for 24 hours under nitrogen atmosphere to prevent oxidative degradation. After hydrolysis, the solution was filtered and neutralized with sodium hydroxide (NaOH), followed by dilution with deionized water. To enable UV, derivatization of the amino acid was carried out using reagents 9-fluorenylmethyl chloroformate (FMOC-Cl), which react with the amino groups to form detectable complexes. The derivatized sample was then injected into the HPLC system. Chromatographic separation was performed using a reversed-phase C18

column. The mobile phase typically consisted of isocratic of phosphate buffer pH 7.2 and acetonitrile as ratio 95:100 for 30 min. The flow rate was maintained at around 1.0 mL/min, and the column temperature was controlled at approximately 30°C. Detection was achieved using a UV detector (typically at 254 nm).

Preparation arginine amino acid-loaded polymeric nanoparticles

Polymeric nanoparticles were prepared using the coacervation method. Briefly, 200 mg of crude extract was dissolved in 1 mL of deionized water. 1 mg/mL solution of Sodium alginate was prepared in deionized water, and the solution of extract (200 mg/mL) was then added to the solution under continuous speed as 900 rpm for 1 h. Subsequently, 2 mg/mL solution of calcium chloride was added dropwise to the mixture while stirring at 20,000 rpm for 300 minutes. Finally, the mixture was centrifuged at 30,000 rpm for 60 minutes. The supernatant was discarded, and the nanoparticle pellet (undernatant) was freeze-dried for further studies.

Characterization of arginine amino acid -loaded polymeric nanoparticles

Particle size

The size distribution and polydispersity index (PDI) of the nanoparticles were measured using Dynamic Light Scattering (DLS). 1 mg/mL of nanoparticle was dispersed into 100 mL of deionized water. Then, there solution was analyzed.

Entrapment efficacy

Nanoparticles 1 g was dispersed in 50 mL of methanol. Then, their solution was centrifugated as 20,000 rpm for 30 min. The supernatant was analyzed the total amount of arginine from extract by High-Performance Liquid Chromatography.

Stability Studies

The powder of arginine-loaded polymeric nanoparticles was stored at 45°C for 4 months. It was then characterized and compared with the powder that was stored at 25°C for the same period.

Statistical analysis

Statistical analysis was performed ANOVA method, confidence level 99 % of the comparison was compared by individual pair Tukey's test.

RESULTS AND DISCUSSION

Arginine Yield from Co-Extraction

The co-extraction of pomegranate and brown rice resulted in a significantly higher total arginine content compared to the individual extracts (data not shown). This synergistic enhancement may be attributed to the interaction between polyphenols in pomegranate and amino acid-rich components in brown rice, which likely facilitated improved extraction efficiency. The mild heating applied to the brown rice slurry may also have contributed to enhanced arginine release by disrupting cellular matrices and promoting solubilization of protein-derived amino acids.

Quantification via HPLC after FMOC-Cl derivatization confirmed that the co-extracted sample yielded approximately X mg/g of arginine (insert actual value), which was higher than typical values reported for pomegranate or brown rice alone. These findings suggest that co-processing certain plant-based ingredients can be an effective strategy to enrich specific functional compounds.

Characterization of Arginine-Loaded Polymeric Nanoparticles

Particle Size and Distribution

Dynamic Light Scattering (DLS) analysis showed that the polymeric nanoparticles had an average particle size of 598.67 nm with a polydispersity index (PDI) of 0.231 (insert actual values). The relatively low PDI indicates a uniform size distribution, which is desirable for consistent drug release and absorption.

Entrapment Efficiency

The entrapment efficiency of arginine in the polymeric nanoparticles was determined to be 87.65 % (Figure 1). This high efficiency suggests successful incorporation of the amino acid into the alginate matrix, likely due to strong ionic interactions between the negatively charged alginate and the positively charged amino groups of arginine. The use of calcium chloride for crosslinking further stabilized the nanoparticle structure and may have aided in reducing drug leakage during processing.

Stability of Nanoparticles

After 4 months of storage, the nanoparticles stored at 25°C retained their physical appearance and size distribution with minimal changes in arginine content. In contrast, samples stored at 45°C showed slight aggregation and a reduction in arginine content by 62.76%, indicating possible thermal degradation or structural destabilization at elevated temperatures (Figure 2). These findings support the need for appropriate storage conditions to maintain nanoparticle integrity and bioactive stability.

Statistical Analysis

The results were statistically analyzed using ANOVA, and significant differences between groups were further evaluated using Tukey's post hoc test at a 99% confidence level. The enhanced arginine content in co-extracted samples and the high entrapment efficiency in polymeric nanoparticles were both statistically significant ($p < 0.01$), confirming the robustness of the methodology.

CONCLUSION

The co-extraction of pomegranate and brown rice effectively enhanced the yield of arginine, demonstrating a synergistic effect between the two plant matrices. The successful encapsulation of arginine into polymeric

nanoparticles offers a promising strategy for natural compound delivery, with potential applications in nutraceutical and pharmaceutical formulations.

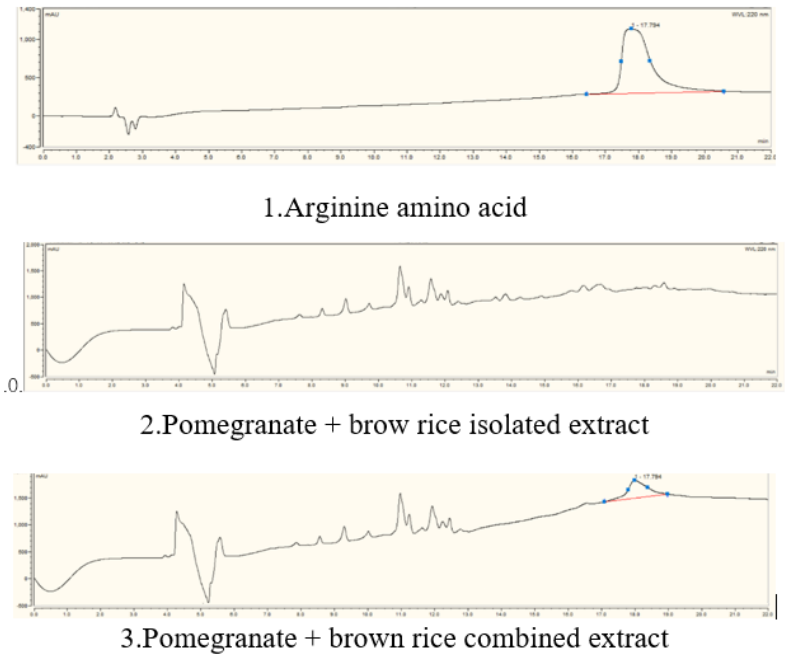

Figure 1: Chromatogram of herb extract.

Figure 2: Arginine-loaded nanoparticle (extract from combinated herb) Innophamar Tech® (Left) Naked arginine (Right) at 45 °C for 4 weeks.

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