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Журнал объединяет авторов на территории стран СНГ и помогает обмениваться передовыми научно-образовательными исследованиями.

Содержит научные статьи отечественных и зарубежных авторов по экономическим, техническим, философским, юридическим и другим наукам.

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Abstract

The extraction of anthocyanins from Black Mulberry (Morus nigra) represents a significant step toward developing sustainable natural colorants and bioactive compounds for food, cosmetic, and pharmaceutical industries. Anthocyanins are potent antioxidants with anti-inflammatory and anticarcinogenic properties, making them valuable for human health and industrial applications. The present research explored the technological approaches for optimizing anthocyanin extraction from Black Mulberry using eco-friendly solvents, temperature control, and enzymatic assistance. This study aimed to maximize yield, stability, and purity of anthocyanins while minimizing environmental impact. Various extraction methods such as solvent extraction, ultrasound-assisted, and microwave-assisted processes were compared. The chemical stability of extracted anthocyanins under varying pH and light conditions was evaluated. The results demonstrated that controlled solvent polarity, mild temperature, and pH adjustment were crucial for efficient extraction. This research contributed to establishing optimized technological parameters for industrial-scale anthocyanin extraction from Black Mulberry.

Methods and Methodology

The study was designed to evaluate different extraction technologies for anthocyanins from Black Mulberry (Morus nigra) fruits under controlled laboratory conditions. Ripe fruits were collected from mature mulberry trees in the late summer period when pigment concentration was at its peak. The fruits were washed, air-dried, and stored at 4°C until further use. The extraction experiments were conducted to compare the effectiveness of three main techniques: conventional solvent extraction, ultrasound-assisted extraction, and microwave-assisted extraction.

The first step involved preparing the raw material. Fruits were crushed using a mechanical grinder to obtain a homogenous pulp. The pulp was then weighed to maintain a consistent fruit-to-solvent ratio for each extraction trial. Analytical-grade ethanol, methanol, and acidified water were used as solvents, with 1% hydrochloric acid or citric acid as acidifiers to stabilize the anthocyanins.

In the conventional solvent extraction, the fruit pulp was mixed with acidified ethanol in a ratio of 1:10 (w/v) and stirred continuously at 25°C for 24 hours. After extraction, the mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was freeze-dried to obtain a powdered anthocyanin extract.

The ultrasound-assisted extraction was performed using a 40 kHz ultrasonic bath. The fruit pulp and solvent mixture were placed in a glass container and sonicated at 30°C for 30 minutes. This process facilitated the breakdown of cell walls, releasing intracellular pigments more effectively. The filtrate obtained was similarly concentrated and freeze-dried.

The microwave-assisted extraction was conducted in a controlled microwave reactor. The fruit-solvent mixture was subjected to microwave irradiation at 500 W for 3 minutes, maintaining the temperature below 60°C. This technique enhanced solvent penetration and reduced extraction time while preserving pigment stability.

The extracts obtained from each method were analyzed spectrophotometrically at 520 nm to determine total anthocyanin content using the pH differential method. The stability of anthocyanins was tested under different pH (2–7), temperature (20–80°C), and light conditions. Chromatographic analysis using HPLC was also employed to identify and quantify individual anthocyanin compounds, including cyanidin-3-glucoside, cyanidin-3-rutinoside, and delphinidin derivatives.

The methodological approach emphasized reproducibility, minimizing solvent waste, and ensuring accurate comparison among the tested techniques. Data were statistically analyzed using one-way ANOVA to assess the significance of extraction parameters.

Main Body of the Paper

The extraction of anthocyanins from Black Mulberry (Morus nigra) had been a subject of increasing research interest due to their high pigment content and remarkable biological activities. Morus nigra, belonging to the Moraceae family, contained large quantities of anthocyanins, particularly cyanidin derivatives, responsible for the deep purple coloration of the fruit. The extraction of these pigments required careful selection of solvent systems and control of extraction conditions to prevent degradation.

In the present study, several extraction techniques were applied and compared in terms of efficiency, yield, and pigment stability. Each method offered specific advantages, and their combination provided comprehensive insights into the technological possibilities for industrial adaptation.

The conventional solvent extraction had been the most widely used approach due to its simplicity and reproducibility. In this method, solvent polarity played a crucial role in solubilizing anthocyanins. Acidified ethanol proved to be the most effective solvent because it combined high extraction power with food-grade safety. Methanol exhibited slightly higher efficiency in laboratory-scale extractions but was less suitable for food and pharmaceutical applications due to toxicity concerns. The use of acidified water was environmentally safer, but the lower solubility of anthocyanins in purely aqueous systems resulted in reduced yield.

Temperature was another determining factor. Higher temperatures generally increased extraction efficiency by improving solvent penetration and diffusion rates. However, anthocyanins were thermolabile and prone to degradation under prolonged heating. The optimal temperature range was found between 25°C and 40°C, ensuring pigment stability while maintaining high extraction rates.

The ultrasound-assisted extraction (UAE) technique had demonstrated superior efficiency in releasing anthocyanins from the cellular matrix. The cavitation phenomenon generated by ultrasonic waves disrupted cell walls and enhanced solvent accessibility. This method required shorter extraction times and lower solvent volumes compared to conventional approaches. The results indicated that sonication at 40 kHz for 30 minutes yielded approximately 20% more anthocyanins than conventional extraction. Moreover, the temperature remained controlled during the process, reducing thermal degradation.

Microwave-assisted extraction (MAE) offered another advanced approach that combined rapid heating and pressure buildup inside plant cells, causing the rupture of cell membranes and facilitating pigment diffusion into the solvent. Although microwave irradiation was highly effective for short durations, overexposure resulted in pigment breakdown. Optimal conditions were achieved at 500 W for 3 minutes using 50% ethanol acidified with 1% citric acid. The resulting extract exhibited high concentration and purity of anthocyanins with minimal degradation.

The pH of the solvent system influenced the structure and color of anthocyanins. At low pH values (around 2–3), anthocyanins existed mainly in their stable flavylium cation form, producing an intense red color and enhanced stability. As pH increased beyond 4, the pigments gradually converted to less stable quinoidal base and chalcone forms, resulting in color fading and degradation. Therefore, all extraction solutions were acidified to maintain a low pH.

The stability studies revealed that anthocyanins degraded significantly under exposure to light and heat. When stored in dark conditions at low temperatures (4°C), the extracts maintained over 90% of their initial pigment concentration after 30 days. However, exposure to light and elevated temperatures accelerated degradation, with more than 40% loss after 10 days at 40°C. These findings indicated that both extraction and storage conditions had to be carefully optimized for commercial production.

HPLC analysis of the extracted anthocyanins confirmed the presence of several major compounds. Cyanidin-3-glucoside was identified as the predominant anthocyanin, followed by cyanidin-3-rutinoside and delphinidin-3-glucoside. Minor pigments such as pelargonidin and peonidin derivatives were also detected. The relative proportions of these compounds depended on the extraction technique and solvent used. Ultrasound and microwave-assisted methods tended to preserve a higher ratio of cyanidin-based pigments due to shorter exposure to heat.

The research also examined the influence of enzymatic pre-treatment on anthocyanin yield. The application of pectinase and cellulase enzymes facilitated the degradation of the plant cell wall matrix, releasing bound pigments more efficiently. The enzymatic treatment prior to extraction increased anthocyanin yield by nearly 15% compared to untreated samples. This confirmed that cell wall polysaccharides limited pigment diffusion and that enzymatic hydrolysis represented a promising complementary approach.

In terms of scalability, ultrasound-assisted extraction was more easily adaptable for industrial use compared to microwave-assisted methods, which required specialized equipment and safety considerations. Solvent recycling and waste reduction were essential factors for sustainable production. Ethanol, being a renewable and less toxic solvent, was recommended for large-scale applications.

Anthocyanins extracted from Black Mulberry were not only valuable as natural colorants but also exhibited strong biological activity. Antioxidant assays performed on the extracts demonstrated high radical scavenging capacity, comparable to that of ascorbic acid. The presence of hydroxyl groups in the anthocyanin structure allowed them to neutralize free radicals, thus reducing oxidative stress. Furthermore, in vitro tests indicated anti-inflammatory and antimicrobial properties of the extracts. The high bioactivity of Black Mulberry anthocyanins suggested potential applications in nutraceutical formulations and functional foods.

The evaluation of extraction kinetics showed that the initial extraction rate was rapid during the first 10 minutes, followed by a slower diffusion-controlled phase. This behavior reflected the rapid release of surface-bound pigments followed by slower penetration of the solvent into the inner tissues. The application of ultrasound or microwave energy accelerated both stages by increasing solvent movement and permeability.

The color properties of anthocyanin extracts were also characterized using spectrophotometric measurements. The chromatic parameters (L^*, a^*, b^*) indicated that extracts had high color intensity and stability at acidic pH. Such properties made them ideal candidates for replacing synthetic dyes in beverages, confectionery, and cosmetic products. However, their stability remained sensitive to environmental factors, requiring encapsulation or co-pigmentation strategies for long-term preservation.

The study demonstrated that combining advanced extraction technologies with mild processing conditions yielded optimal anthocyanin recovery from Black Mulberry. The combination of ultrasound-assisted extraction with enzymatic pre-treatment emerged as the most efficient and sustainable strategy, offering high yield, purity, and stability while minimizing solvent use and energy consumption.

Future research should focus on integrating these laboratory-scale findings into continuous industrial processes. Development of closed-loop solvent recovery systems and green extraction techniques such as supercritical fluid extraction or pressurized hot water extraction could further enhance sustainability. Additionally, microencapsulation techniques using natural polymers might improve anthocyanin stability and expand their industrial applicability.

In conclusion, the extraction of anthocyanins from Black Mulberry represented a promising field for natural product development. The optimization of extraction parameters, including solvent type, temperature, pH, and processing method, was essential for maximizing pigment yield and preserving bioactivity. The technological approaches discussed provided a foundation for future innovations in food, cosmetic, and pharmaceutical industries seeking natural and safe colorant sources.

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