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RESEARCH ARTICLE

Mechanistic Insights into the Extraction and Stabilization of Betalains and Phenolic Compounds from Beetroot

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Abstract

Beetroot (*Beta vulgaris* L.) has gained significant attention as a functional food due to its high content of bioactive compounds, including betalains, phenolic acids, flavonoids, and nitrates, which contribute to antioxidant, anti-inflammatory, and cardioprotective effects. Despite their health potential, these compounds face significant challenges due to chemical instability and poor bioavailability, exacerbated by environmental and physiological factors such as light, temperature, pH, and digestive degradation. Recent advances in extraction science have focused on optimizing yield, selectivity, and compound stability through innovative and environmentally friendly approaches. Techniques such as ultrasound-assisted (Table 2), microwave-assisted (Table 3), enzyme-assisted, and pressurized liquid extraction have demonstrated improved efficiency and reduced solvent usage compared to conventional methods. Furthermore, the use of green solvents such as deep eutectic solvent systems has shown remarkable potential for selectively extracting and stabilizing betalains and phenolics. Emerging encapsulation technologies, such as nanoemulsions, polymeric nanoparticles, and cyclodextrin inclusion complexes, further enhance bioavailability by protecting beetroot bioactives during digestion and promoting controlled release. This review provides a critical synthesis of these technological developments, emphasizing the relationship between extraction efficiency, compound stability, and biological accessibility. It also highlights remaining challenges and perspectives for translating laboratory-scale innovations into industrial applications to support the development of stable, bioavailable, and sustainable beetroot-based nutraceuticals and functional foods.

Keywords: Beetroots, Betalains, Bioavailability, Green Extraction, Nutraceuticals, Stability.

1. Introduction

Beetroot (*Beta vulgaris* L.) has emerged as a valuable source of natural bioactive compounds and colorants, offering significant health and nutritional benefits that extend beyond its culinary use. It is particularly rich in betalains, comprising betacyanins and betaxanthins, as well as phenolic acids, flavonoids, ascorbic acid, and dietary nitrates, which together contribute to its antioxidant, anti-inflammatory, anticarcinogenic, and cardiovascular-protective properties (Sadowska-Bartosz & Bartosz, 2021). Owing to these multifunctional attributes, beetroot and its derivatives have been increasingly incorporated into functional foods, dietary supplements, and natural health products (Stoica et al., 2025). However, the large-scale application of beetroot bioactives remains hindered by their chemical instability and low bioavailability (Bian et al., 2024). Betalains are highly sensitive to environmental factors such as light, temperature, oxygen, and pH fluctuations, which can cause pigment degradation and loss of bioactivity during processing, storage, and digestion (Yeasmen et al., 2025). These challenges necessitate the development of innovative extraction and stabilization strategies to preserve bioefficacy and improve absorption in the human body.

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Traditional extraction methods, particularly solvent-based methods, are often limited by high energy consumption, low selectivity, and potential degradation of heat-labile compounds. In response, recent studies have focused on modern, eco-efficient extraction techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), and pressurized liquid extraction (PLE), which significantly improve mass transfer, reduce solvent usage, and enhance recovery yields (Mungwari et al., 2025; Sun et al., 2025).

Moreover, green solvents, such as deep eutectic solvents (DESs) composed of biodegradable components, including choline chloride, organic acids, and polyols, have demonstrated superior extraction efficiency and pigment stability compared to conventional organic solvents. These advances align with the principles of green chemistry and sustainability, promoting environmentally responsible and energy-efficient bioprocessing (Chevé-Kools et al., 2025).

Beyond extraction, emerging encapsulation and nanodelivery technologies, including polymeric nanoparticles, liposomes, nanoemulsions, and cyclodextrin inclusion complexes, offer new opportunities to improve the stability, solubility, and intestinal bioavailability of beetroot-derived compounds (Huang et al., 2025). Collectively, these innovations represent a significant step toward transforming beetroot extracts into robust functional ingredients for food, cosmetic, and nutraceutical applications. This review aims to synthesize recent advancements in extraction and stabilization strategies for beetroot bioactives, discuss their impact on compound integrity and bioavailability (Figure 1), and identify research gaps and industrial challenges that must be addressed to achieve scalable and sustainable production.

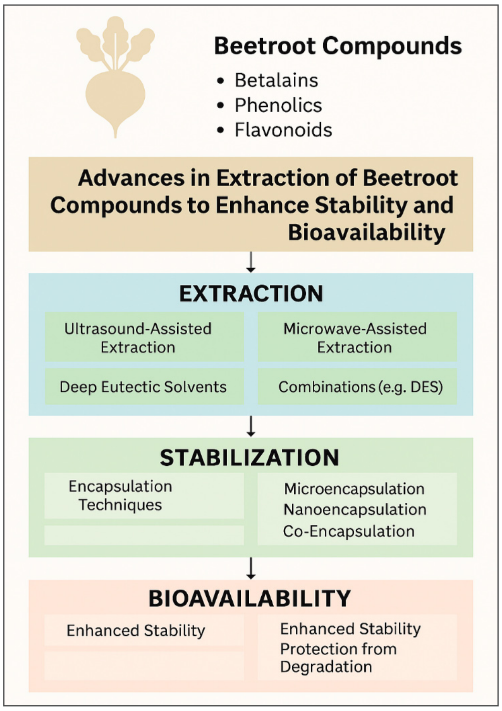

Figure 1. *Schematic representation of the extraction, stabilization, and bioavailability enhancement pathways for beetroot (Beta vulgaris L.) bioactive compounds.*

2. Conventional Solvent Extraction of Beetroot Bioactives

Conventional solvent extraction (CSE) has long served as the foundation for isolating bioactive constituents from botanical materials, including *Beta vulgaris* L. (red beetroot). This method relies on immersing comminuted plant tissue in a solvent system that dissolves target compounds through diffusion and mass-transfer processes driven by concentration gradients (Zin et al., 2020). In beetroot, CSE is primarily employed to recover betalains, phenolic acids, flavonoids, ascorbic acid, and sugars that collectively determine the vegetable's color, antioxidant capacity, and health benefits. Due to its simplicity, modest equipment needs, and reproducibility, CSE remains the benchmark for comparing efficiency and selectivity among emerging extraction techniques (Table 1). Nevertheless, increasing environmental and industrial demands for sustainability have revealed the main shortcomings in this conventional approach, motivating a wave of methodological innovation.

Table 1. *Overview of Conventional Solvent Extraction (CSE) of Beetroot Bioactives*

Parameter	Typical Range / Description	Advantages	Limitations	References
Solvent systems	Water, ethanol–water (50–70%), methanol, acetone	Simple, inexpensive, food-grade solvents available	High solvent use, residual toxicity (non-food solvents)	Ghareaghajlou et al., 2021
Temperature / time	25–60 °C for 1–3 h	Moderate heating enhances diffusion	Pigment degradation above 60 °C	Delgado-Vargas et al., 2000
Solid–liquid ratio	1:10 – 1:25 (w/v)	Adjustable for scalability	Excess solvent increases cost	Alagu et al., 2018
Optimization factors	pH 5–6, agitation 300 rpm	Controlled pH preserves betalains	Energy-intensive evaporation	Chemat et al., 2019
Key outcome	Betalain yield ≈ 10–15 mg g^{-1} DM	Established baseline method	Low selectivity, oxidative losses	Kaba et al., 2024

2.1 Solvent Selection and Polarity Considerations

The polarity of the solvent governs solubility and selectivity for different classes of beetroot phytochemicals. Polar protic solvents such as water, ethanol, and methanol are most frequently employed because betalains and phenolic acids contain multiple hydroxyl and carboxyl groups that form hydrogen bonds with these solvents (Lama-Muñoz & Contreras, 2022). Ethanol–water mixtures (50–70 %, v/v) often yield the highest betalain recovery because they balance polarity with viscosity and diffusion properties (Eyshi et al., 2024). However, solvent toxicity and food-grade regulations restrict the use of methanol for nutraceutical applications, prompting a shift toward ethanol and water as safer alternatives (Karageorgou et al., 2025).

Co-solvent systems have been developed to modulate polarity and enhance mass transfer. For example, the inclusion of small proportions of acetone or acetic acid can improve penetration into plant matrices but may also accelerate pigment degradation if pH becomes too low. Betalains are particularly unstable under strongly acidic (pH < 4) or alkaline (pH > 8) conditions due to the cleavage of the imine bond linking betalamic acid to cyclo-DOPA (Cejudo-Bastante et al., 2016). Therefore, maintaining near-neutral pH during extraction is critical for pigment integrity. Temperature likewise influences solubility and kinetic rates: moderate heating (30–40 °C) improves diffusion, but excessive heat (>60 °C) promotes oxidation and decarboxylation reactions that bleach the extract (Bahriye et al., 2023).

2.2 Mass-Transfer Mechanism and Kinetics

In conventional maceration, solvent penetration and diffusion across cell walls are rate-limiting steps. Initially, readily accessible surface pigments dissolve quickly, followed by a slower diffusion phase from deeper tissue layers. The driving force for solute migration diminishes as concentration equilibrium approaches, resulting in diminishing returns over time. Stirring, shaking, or mechanical agitation accelerates boundary-layer renewal and shortens extraction time, yet industrially this is often constrained by energy costs (Eko et al., 2025).

Table 2. *Summary of Ultrasound-Assisted Extraction (UAE) of Beetroot Bioactives.*

Operational Parameter	Typical Value / Condition	Advantages	Challenges / Limitations
Ultrasonic frequency	20–45 kHz (bath or probe)	Rapid cell disruption via cavitation	Excess power may oxidize pigments
Power density	100–200 W cm^{-2}	Shorter extraction time (≤ 30 min)	Requires cooling to control heat
Temperature	30–40 °C	Preserves thermolabile compounds	Cavitation decreases above 60 °C
Solvent	50–70 % ethanol or water	Low toxicity, efficient pigment solubilization	Slightly lower yields for non-polar compounds
Performance	↑ Yield 25–35 % vs CSE; ↑ antioxidant activity 20 %	Energy-saving, scalable	Uniform cavitation difficult at large scale

Fick's second law is typically used to model mass-transfer kinetics in solvent extraction, where effective diffusion coefficients depend on particle size, solvent viscosity, and temperature. Reducing beetroot particle size from 2 mm to 0.5 mm can increase pigment yield by more than 30 % but also raises the risk of oxidation due to greater surface exposure (Huang et al., 2018). Vacuum or inert-gas atmospheres can mitigate oxidative losses, though these add processing complexity. Kinetic studies generally show that 70–80% of the total betalains are extracted within the first 90 minutes under optimized conditions; beyond this period, pigment degradation offsets additional yield gains (Kumar et al., 2023).

2.3 Advantages and Limitations of CSE

The main advantages of CSE lie in its operational simplicity, scalability, and minimal equipment requirements. Industrial facilities can readily adapt solvent extraction tanks and rotary evaporators without significant capital investment. Moreover, the technique is compatible with food-grade solvents and does not require specialized training. However, these benefits are counterbalanced by several limitations.

First, low selectivity results in the co-extraction of sugars, pectins, and proteins, complicating downstream purification. Second, high solvent consumption, that is often exceeding 20 L per kg of raw material, leads to substantial recovery and disposal costs (Cheremisinoff, 1995). Third, thermal and oxidative degradation of betalains during extended extraction or solvent evaporation markedly reduces pigment retention. Finally, conventional organic solvents pose environmental and safety concerns; ethanol production and distillation are energy-intensive, and solvent emissions contribute to volatile organic compound (VOC) pollution.

Comparative studies have demonstrated that advanced techniques, such as ultrasound- or microwave-assisted extraction, can double pigment yield while cutting solvent use in half (Cardoso-Ugarte et al., 2014; Fu et al., 2020). Nonetheless, CSE remains indispensable for baseline evaluation, process validation, and large-scale pigment standardization.

2.4 Process Optimization and Statistical Modeling

Modern optimization strategies employ response surface methodology (RSM) or design of experiments (DoE) to systematically assess the interactive effects of temperature, solvent ratio, time, and pH on yield and antioxidant activity. Yong et al. (2018) applied a central composite design to identify optimal conditions of 35 °C, 60 % ethanol, and 1:20 (w/v) solid-to-liquid ratio, yielding 14.2 mg betacyanin g^{-1} dry matter with maximal color stability. RSM models enable predictive control and reduce the number of experimental runs, facilitating industrial scalability.

Kinetic models, such as Peleg's equation and pseudo-second-order models, have also been applied to describe betalain extraction, providing insights into diffusion coefficients and rate constants under varying solvent compositions. Combining these models with spectrophotometric monitoring of absorbance at 538 nm allows real-time assessment of pigment release and degradation kinetics (Milićević et al., 2021).

Table 3. *Summary of Microwave-Assisted Extraction (MAE) of Beetroot Bioactives.*

Process Variable	Typical Range / Setting	Advantages	Limitations / Cautions
Microwave frequency / power	2.45 GHz; 300–600 W	Rapid volumetric heating, uniform energy transfer	Hot-spot formation may degrade betalains
Extraction time	3–6 min	Short process cycle	Overheating beyond 7 min reduces color
Solvent	40–60 % ethanol or DES mixtures	Improved diffusion, reduced solvent use	Requires dielectric-loss matching
Temperature	50–65 °C	Enhanced pigment release	Degradation > 70 °C
Outcome	Yield ↑ 40–90 %; energy ↓ 45–60 % vs CSE	Efficient, green, scalable	Capital cost, uniformity control

To further enhance efficiency, some researchers have incorporated pre-treatments, such as blanching, osmotic dehydration, or enzyme-assisted cell-wall degradation, to improve solvent accessibility (Asghrai et al., 2024; Zeng et al., 2025). Enzymes such as pectinase or cellulase partially hydrolyze cell wall polysaccharides, increasing pigment diffusivity, although enzyme costs and thermal sensitivity limit their widespread use in conventional setups.

2.5 Purification and Concentration Steps

Following extraction, the crude beetroot solution typically contains pigments, sugars, organic acids, and residual solvent. Filtration and centrifugation remove insoluble material, after which rotary evaporation or membrane concentration is used to recover solvent and increase pigment concentration. Adsorption techniques using ion-exchange resins or activated carbon can selectively purify betalains, but the cost of regeneration and pigment losses remain concerns (Akhtar et al., 2024). Freeze-drying is often preferred over spray-drying because it preserves color and antioxidant activity, though it requires longer processing times and higher energy input. The purified extracts can then be standardized by high-performance liquid chromatography (HPLC) or spectrophotometric methods to ensure consistent pigment composition before downstream formulation.

2.6 Environmental and Economic Aspects

From a sustainability perspective, conventional solvent extraction is energy-intensive and generates significant solvent waste. Life-cycle assessments show that solvent recovery and distillation account for 60–70 % of total process energy (Capello et al., 2005). Transitioning to renewable ethanol sources and implementing closed-loop solvent-recycling systems can reduce environmental impact but require capital investment. In contrast, water-based extractions, though environmentally benign, yield lower pigment concentrations and are prone to microbial spoilage if not immediately stabilized. Hybrid solvent systems incorporating glycerol or propylene glycol, both GRAS-classified compounds, offer a compromise between efficiency and safety (Padmawar & Bhadoriya, 2018; Mani et al., 2025).

Economically, solvent cost and recovery efficiency largely determine process viability. Industrial beetroot pigment producers often accept moderate yield reductions in exchange for lower solvent consumption and simplified purification, underscoring the trade-offs inherent in conventional extraction processes. Integration of CSE with valorization of residual beet pulp (as fiber or biofertilizer) can further improve economic sustainability.

2.7 Outlook and Technological Transition

Although conventional solvent extraction remains dominant in industrial pigment production, future developments are expected to shift toward hybrid and green extraction systems that combine traditional maceration with physical enhancements such as ultrasound, microwaves, or pulsed electric fields. These approaches can significantly shorten extraction time and solvent usage without fundamentally altering existing infrastructure (Kaba et al., 2024). Additionally, regulatory trends favor natural and sustainable processing methods, compelling manufacturers to adopt greener solvents, such as deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs), that meet food-safety requirements (Mišan et al., 2020).

Research is also expanding toward process intensification, continuous counter-current extraction, membrane-assisted solvent recovery, and real-time colorimetry-based control systems to improve reproducibility and scalability. Nevertheless, the empirical knowledge derived from decades of conventional solvent extraction underpins all these innovations. As such, CSE continues to provide essential reference data to understand diffusion mechanisms, solvent–solute interactions, and degradation pathways, thereby guiding the rational design of advanced extraction technologies.

3. Ultrasound-assisted Extraction (UAE) of Beetroot Bioactives

Ultrasound-assisted extraction (UAE) is widely recognized as one of the most effective modern green extraction techniques for recovering bioactive compounds from plant matrices, including *Beta vulgaris* L. (red beetroot) (Singh et al., 2025). The method utilizes acoustic cavitation, including the formation, growth, and implosive collapse of microbubbles in a liquid medium, to enhance mass transfer between the solvent and plant tissues. These rapid mechanical and thermal effects disrupt cell walls, increase solvent penetration, and release intracellular constituents with minimal heat exposure (Vyas et al., 2019). As betalains and phenolics are sensitive to thermal degradation, UAE offers significant advantages over conventional solvent extraction by shortening processing time, reducing solvent usage, and improving pigment stability and antioxidant retention (Bashir et al., 2025). The technique has become increasingly relevant in the context of sustainable bioprocessing, aligning with the principles of green chemistry and circular-economy manufacturing.

3.1 Principles of Acoustic Cavitation and Mechanistic Insights

The core mechanism behind UAE lies in acoustic cavitation. When ultrasonic waves (typically 20–100 kHz) pass through a liquid, alternating high- and low-pressure cycles cause the formation of microbubbles. Their subsequent collapse generates localized “hot spots” with transient temperatures approaching 5,000 K and pressures near 1000 atm (Vinatoru, 2015). Although these extreme conditions occur on a microsecond timescale, they create strong shear forces and shock waves that mechanically disrupt plant cells and improve solvent infiltration. In beetroot tissue, this results in efficient rupture of the parenchymal cells containing vacuolar betalains, enabling rapid pigment leaching into the solvent phase.

These effects occur without significantly increasing bulk temperature, which is critical for preserving thermolabile pigments such as betanin and isobetanin (Delgado-Vargas et al., 2000). The agitation produced by ultrasonic streaming also thins the boundary layer around plant particles, reducing diffusion resistance and enhancing solute transfer (Chemat et al., 2019). Studies using scanning electron microscopy (SEM) confirm that ultrasound induces pronounced structural deformation of beetroot cell walls and the middle lamella, corroborating the mechanical basis for enhanced extraction (Kaba et al., 2024).

3.2 Optimization of Operational Parameters

The efficiency of the UAE is governed by multiple factors, including ultrasonic frequency, power intensity, extraction time, temperature, and solvent composition. Lower frequencies (20–40 kHz) generate more violent cavitation and greater mechanical disruption, whereas higher frequencies produce more uniform but less intense cavitation bubbles (Chuai et al., 2024). For beetroot extraction, frequencies between 35 and 45 kHz, combined with power densities of 100–200 W cm^{-2}, yield optimal results, achieving high pigment recovery with minimal degradation (Tutunchi et al., 2019).

Temperature plays a dual role: moderate heating (30–40 °C) enhances solvent diffusion and cavitation dynamics, whereas higher temperatures (>60 °C) decrease cavitation intensity because vapor pressure dampens bubble collapse (Chemat et al., 2019). Therefore, mild-temperature conditions are preferred to protect betalain integrity. Extraction duration typically ranges from 10 to 40 minutes; that is, more prolonged exposure increases yield up to an optimum, after which pigment degradation and radical oxidation outweigh extraction gains (Kaba et al., 2024).

Solvent type and polarity remain crucial. Ethanol–water mixtures (50–70 %) or acidified aqueous ethanol at pH 6–7 provide superior solvating capacity for both hydrophilic and moderately polar compounds. Solvent-to-solid ratios of 15–25 mL g^{-1} are often reported as optimal, balancing mass transfer with solvent economy (Lazar et al., 2021). Statistical optimization using response-surface methodology (RSM) has confirmed that interactions among these parameters significantly affect yield and color stability. Shofinita et al. (2023) demonstrated that applying UAE at 40 kHz for 25 minutes with 60 % ethanol increased total betalain content by 1.5-fold compared with conventional extraction, while reducing energy consumption by 40 %.

3.3 Comparative Performance and Kinetic Modeling

Quantitative comparisons consistently show that UAE outperforms maceration and Soxhlet extraction in both yield and pigment preservation. Betanin recovery can increase by 25–35% and total phenolic content by 20–30%, depending on conditions (Tutunchi et al., 2019; Smirani et al., 2025). Moreover, antioxidant assays (DPPH, ABTS, FRAP) demonstrate higher radical-scavenging activity in UAE extracts, reflecting improved retention of bioactives.

Kinetic modeling using Peleg's equation and second-order diffusion models reveals that UAE markedly increases the apparent mass-transfer coefficient, effectively reducing extraction equilibrium time (Delgado-Vargas et al., 2000). The pseudo-first-order rate constants (k_1) for betalain extraction under ultrasound are approximately double those for conventional maceration. These data support the mechanistic premise that cavitation accelerates both solvent penetration and solute diffusion.

Energy-efficiency analyses indicate that ultrasound reduces overall energy input per unit of pigment yield by 30–50 % relative to thermal or mechanical methods (Linares & Rojas, 2022). This improved efficiency contributes to the UAE's environmental sustainability and justifies its rapid adoption in the natural-colorant industry.

3.4 Integration with Green Solvents and Emerging Hybrid Systems

The combination of ultrasound with green solvents, particularly deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs), has recently gained prominence. These solvents, composed of biodegradable constituents such as choline chloride, lactic acid, or glycerol, exhibit tunable polarity and strong hydrogen-bonding capacity that stabilize betalain structures during extraction (Abbott et al., 2004; Kaba et al., 2024). Kaba et al. (2024) reported that coupling UAE with a choline chloride–lactic acid DES resulted in a 42% higher total betalain yield and improved pigment stability compared with ethanol–water UAE. The synergistic effect arises from enhanced cell disruption by ultrasound and increased pigment solubility in DESs.

Other hybrid approaches integrate UAE with microwave-assisted heating (UAME) or enzyme-assisted pretreatment. In UAME, ultrasound promotes cavitation-induced mixing while microwaves provide uniform heating, resulting in faster kinetics and reduced solvent use (Singla & Sit, 2021). Enzyme-ultrasound coupling (UAE-EAE) uses pectinase or cellulase to weaken cell walls before ultrasonic treatment, thereby improving the release of bound phenolics. These hybrid systems exemplify process intensification strategies that can be tailored to specific compound profiles.

3.5 Quality, Stability, and Analytical Characterization

Maintaining pigment quality and antioxidant activity is essential for functional-food applications. Spectroscopic analyses indicate that UAE extracts preserve the characteristic absorption maxima of betanin (538 nm) and betaxanthin (480 nm), confirming minimal structural alteration (Delgado-Vargas et al., 2000). High-performance liquid chromatography (HPLC) profiles further show that ultrasound does not induce significant isomerization or decarboxylation of betalains when performed under controlled temperature.

Colorimetric measurements (CIE L*a*b*) indicate higher chroma and lower hue variation in UAE extracts compared with thermally processed samples, suggesting superior color stability (Wu et al., 2022). Total antioxidant capacity (measured via DPPH and FRAP assays) remains 10–20 % higher after 15 days of refrigerated storage, likely due to the absence of prolonged heat exposure (Sonawane & Arya, 2014). These findings underscore the UAE's ability to produce high-quality extracts suitable for downstream encapsulation and formulation.

3.6 Industrial Scalability and Equipment Design

Although the UAE has proven effective at the laboratory scale, its industrial implementation poses engineering challenges. Scale-up from batch ultrasonic baths to continuous-flow or probe systems requires careful control of acoustic field distribution to avoid dead zones where cavitation intensity is low (Dong et al., 2020). Modern designs employ multiple transducers, flow-through reactors, and cooling jackets to ensure uniform cavitation while maintaining temperature below 40 °C.

Power consumption on an industrial scale is another critical factor. Energy requirements increase exponentially with volume if acoustic intensity is not optimized. However, recent pilot-plant studies have demonstrated that using flow-through reactors with 1–2 kW transducers can process up to 100 L h^{-1} of beetroot slurry while maintaining consistent pigment yield (Silva et al., 2020). Integration with solvent-recycling loops further improves process economy. Given its compatibility with ethanol–water mixtures and absence of toxic residues, UAE aligns well with food-industry regulations and clean-label requirements.

3.7 Environmental and Economic Considerations

From a sustainability standpoint, the UAE represents a significant advance over traditional extraction methods. The technique reduces solvent use, extraction time, and energy input, resulting in lower greenhouse gas emissions and operational costs. Life-cycle analyses have shown that ultrasound-based processes emit 40–60 % less CO_2-equivalent per kilogram of pigment than conventional solvent extraction (Dias et al., 2021). Moreover, using water or ethanol, both renewable and food-grade, minimizes environmental hazards.

Economic analyses suggest that despite higher equipment investment, the UAE achieves cost parity within three years of operation due to savings in solvent and energy consumption (Shofinita et al., 2023). The technology's modularity also allows retrofitting existing extraction lines, reducing the need for complete plant redesign. As demands for natural colorants and functional ingredients continue to rise, the UAE's eco-efficiency offers a competitive advantage for industrial producers of beetroot extracts.

3.8 Limitations and Future Perspectives

Despite its benefits, the UAE is not without limitations. Over-intensified sonication can cause pigment oxidation by generating free radicals in the solvent medium (Linares & Rojas, 2022). Controlling amplitude and duty cycle is thus essential to avoid degradation. Additionally, the process may generate local heating and noise pollution, requiring cooling systems and acoustic insulation.

Future research should focus on integrating real-time monitoring tools, such as in-situ spectrophotometry and acoustic-emission sensors, to optimize cavitation intensity dynamically. Computational fluid dynamics (CFD) modeling of ultrasound reactors can aid in designing scalable geometries that ensure homogeneous cavitation distribution. Hybrid extraction networks that couple UAE with deep eutectic solvents, pulsed electric fields, or supercritical CO_2 are likely to dominate next-generation bioprocessing, providing higher yields, greater stability, and a lower environmental footprint. Finally, standardization of food-grade ultrasonic parameters (frequency, power density, exposure time) is needed for regulatory acceptance and reproducible product quality.

4. Deep Eutectic Solvent (DES) Extraction of Beetroot Bioactives

4.1 Introduction to Deep Eutectic Solvent (DES)

Deep eutectic solvents (DESs) have recently emerged as transformative media for the extraction of natural compounds, offering an eco-friendly alternative to traditional organic solvents. DESs are typically formed by mixing a hydrogen bond acceptor (HBA) such as choline chloride, betaine, or ammonium salts, with a hydrogen bond donor (HBD) such as organic acids, polyols, amides, or sugars, in specific molar ratios. The strong hydrogen-bond interactions between the components lower the mixture's freezing point well below that of the individual components, yielding a stable liquid phase at room temperature (Abbott et al., 2004).

DESs share many physicochemical properties with ionic liquids (ILs), such as negligible vapor pressure, high thermal stability, and tunable polarity, but they are simpler to prepare, biodegradable, and less toxic. They can dissolve a wide range of biomolecules, making them ideal for the recovery of thermolabile pigments, polyphenols, and antioxidants from food matrices (Skulcova et al., 2018). For beetroot (*Beta vulgaris* L.), DESs have proven particularly suitable due to the high polarity of betalains and phenolic compounds (Table 4), which form hydrogen bonds with DES components, enhancing solubility and protecting against oxidation (Kaba et al., 2024).

Table 4. *Deep Eutectic Solvent (DES) Extraction of Beetroot Bioactives*

DES Composition / Parameter	Typical Condition / Example	Advantages	Drawbacks / Challenges
HBA + HBD systems	Choline chloride + lactic acid (1:2); + glycerol (1:2)	Biodegradable, non-volatile, tunable polarity	High viscosity reduces mass transfer
Water content	10–30 % (w/w)	Adjusts viscosity, enhances diffusion	Excess water lowers solvating power
Temperature / time	40–55 °C / 20–40 min	Mild heating preserves pigments	Higher temp > 60 °C → oxidation
Integration	DES + UAE or MAE hybrids	Boosts yield > 40 %, stabilizes betalains	Process complexity
Performance / stability	Up to 23–25 mg g^{-1} DM; color retention > 90 % (30 days @ 4 °C)	High efficiency, recyclable solvent	Limited regulatory data for food use

4.2 Mechanism of DES Action in Extraction

The extraction efficiency of DESs arises from their unique microstructure. Hydrogen-bond networks between HBA and HBD components create a polar, protic environment with high viscosity but strong solvation capability. This environment facilitates electrostatic and hydrogen-bond interactions with hydroxyl, carboxyl, and imine groups of betalains and phenolics, promoting dissolution without requiring high temperature (Abbott et al., 2004).

In beetroot, betalains are zwitterionic molecules with both a positive (imine) and a negative (carboxylate) center. DES systems rich in carboxylic acids or alcohols stabilize these ionic sites through hydrogen bonding, thereby reducing pigment degradation. Choline chloride–lactic acid (1:2 molar ratio) and choline chloride–glycerol (1:2) are among the most effective formulations for betalain extraction because they combine strong hydrogen-bonding capacity with biocompatibility (Kaba et al., 2024).

Moreover, DES viscosity and polarity can be tuned by water dilution (10–30 %) to enhance mass transfer and reduce diffusional resistance. Water acts as a structure-breaker, weakening HBA–HBD interactions and improving solvent mobility, while maintaining sufficient polarity for pigment solvation (Kivela et al., 2022). This tunability enables the selective extraction of hydrophilic betalains versus less-polar phenolic compounds

4.3 Optimization of Extraction Conditions

Optimal DES extraction parameters depend on the HBA–HBD combination, molar ratio, water content, temperature, and extraction time. Studies by Kaba et al. (2024) demonstrated that choline chloride–lactic acid (1:2) at 30 % water content, 50 °C, and 30 min extraction time produced the highest total betalain yield (23.7 mg g^{-1} dry matter), surpassing 70 % ethanol extraction by 40 % and increasing water content beyond 40 % reduced yield due to excessive dilution of DES polarity, while low water (<10 %) increased viscosity and hindered mass transfer.

Response-surface methodology revealed temperature as a key variable: yields rose linearly up to 50 °C but declined at higher temperatures due to betalain instability. Agitation (300–600 rpm) and solid-to-liquid ratios (1:15–1:25 w/v) also significantly affected recovery efficiency (Alagu et al., 2018). Overall, mild heating combined with moderate water dilution offers the best balance between solubility, viscosity, and pigment stability.

4.4 Integration with Ultrasound and Microwave

Because of their high viscosity, DESs can show slow diffusion in static extraction systems. To address this issue, researchers have effectively combined DESs with ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE), forming hybrid "intensified" processes. Ultrasonic cavitation enhances solvent penetration, while microwaves accelerate heating and solubilization in the viscous medium (Vo et al., 2023).

Kaba et al. (2024) reported that UAE-DES extraction using choline chloride–lactic acid with 20 % water at 40 kHz for 25 min increased betalain yield by 42 % compared with DES alone. Similarly, MAE-DES at 360 W for 4 min further boosted pigment recovery by 50%, while maintaining structural integrity, as confirmed by UV–Vis and HPLC analyses. These hybrid systems also demonstrated improved antioxidant activity, with DPPH radical-scavenging capacity exceeding conventional ethanol extracts by 1.3-fold.

The synergy arises from cavitation-induced cell wall disruption (UAE) or dielectric heating (MAE), which counteracts DES viscosity and enhances mass transfer. Combining green solvents with green energy thus represents a paradigm shift toward sustainable bioprocessing.

4.5 Stability, Quality, and Bioactivity of DES Extracts

Beyond yield, DES extraction offers significant advantages in pigment stability and bioactivity retention. The strong hydrogen-bond network in DESs stabilizes betalains against oxidative and photolytic degradation. In storage studies, beetroot DES extracts retained 90% of their color intensity after 30 days at 4 °C, compared with 70% for ethanol extracts (Kaba et al., 2024). The acidic microenvironment (pH 4–5) further suppresses betanin oxidation.

Fourier-transform infrared (FTIR) and UV–Vis spectroscopy confirm that DESs preserve key functional groups of betalains, particularly the C=N and C=O bonds, indicating no significant structural alterations (Aztatzi-Rugerio et al., 2019). HPLC chromatograms show higher proportions of intact betanin and isobetanin, suggesting that DES extraction minimizes decarboxylation and dehydrogenation reactions.

Antioxidant assays (DPPH, ABTS, and FRAP) consistently reveal superior radical-scavenging activity in DES extracts. This improvement reflects both greater recovery of bioactives and potential synergistic effects between DES constituents (e.g., lactic acid, glycerol) and phenolic antioxidants. Additionally, DES extracts exhibit enhanced antimicrobial activity against *E. coli* and *Staphylococcus aureus*, attributed to combined osmotic and oxidative effects (Suriyaprom et al., 2022).

4.6 Environment, Safety, and Economic Aspects

The environmental footprint of DES extraction is markedly lower than that of conventional organic solvents. DESs have negligible volatility, eliminating solvent emissions and explosion hazards (Abbott et al., 2004). Most components, such as choline chloride, lactic acid, and glycerol, are biodegradable and GRAS-certified. The reusability of DESs further enhances sustainability: after extraction, pigments can be precipitated or adsorbed onto resins, enabling solvent recovery via simple filtration and water evaporation. Kaba et al. (2024) achieved 85% solvent recyclability with minimal loss in extraction efficiency after three cycles.

Economically, the low cost and ready availability of DES components make them attractive for industrial adoption. Life-cycle analyses estimate a 40–50 % reduction in operational costs compared with ethanol extraction, mainly due to solvent recycling and lower energy requirements. DES extraction also circumvents the need for expensive solvent recovery systems, such as distillation, simplifying process design (Chemat et al., 2019).

Toxicological safety remains an important consideration. While most natural DESs exhibit low cytotoxicity, their residual presence in food extracts must comply with regulatory standards. Studies on cell viability show that choline chloride-based DESs exhibit minimal toxicity at concentrations below 2 %, indicating their suitability for food-grade pigment applications (Popovic et al., 2023).

5. Factors Affecting the Stability of Beetroot Bioactives

5.1 Overview of Beetroot Bioactives and their Instability

Beetroot (*Beta vulgaris* L.) is renowned for its high concentration of natural pigments and antioxidants. These most notable betalains are unique nitrogen-containing water-soluble pigments responsible for the characteristic red-violet coloration of the root. Betalains are subdivided into betacyanins (e.g., betanin, isobetanin, neobetanin) and betaxanthins (e.g., vulgaxanthin I, miraxanthin V). These compounds exhibit remarkable antioxidant, anti-inflammatory, and hepatoprotective properties (Clifford et al., 2016; Stoica et al., 2025). In addition to betalains, beetroot contains significant levels of phenolic acids (ferulic, caffeic, and p-coumaric acids), flavonoids, ascorbic acid, and dietary nitrates, all of which contribute synergistically to its biological activity (Clifford et al., 2015).

Despite this rich phytochemical profile, the application of beetroot bioactives in food, cosmetic, and pharmaceutical industries is limited by their chemical and physical instability (Table 5). Betalains are highly sensitive to light, temperature, oxygen, metal ions, and pH fluctuations (Yeasmen et al., 2025). Their degradation leads to color fading, loss of antioxidant potential, and reduced bioefficacy. Understanding the physicochemical and biochemical factors influencing betalain stability is thus critical for improving their extraction, formulation, and shelf-life performance.

Table 5. *Factors affecting the stability of beetroot bioactives.*

Stability Factor	Mechanism / Description	Effect on Betalains	Control / Mitigation Strategy
Temperature	Thermal oxidation and decarboxylation above 60 °C	Pigment bleaching, color fading, neobetanin formation	Mild processing (≤ 50 °C), freeze-drying, inert atmosphere
pH	Acidic (pH < 4) → hydrolysis; alkaline (pH > 8) → ring cleavage	Loss of chromophore, reduced antioxidant activity	Maintain pH 5–6 during extraction/ formulation
Oxygen & Light	Photo-oxidation and radical formation	Color degradation, structural cleavage	Nitrogen flushing, opaque packaging
Metal Ions (Fe^{3+}, Cu^{2+})	Catalyze oxidation of betalamic acid moiety	Precipitation, browning	Add chelators (EDTA, citric acid)
Water Activity / Matrix	High a_w and oxygen diffusion accelerate reactions	Faster pigment loss	Reduce moisture, use biopolymer matrices

5.2 Chemical Structure and Degradation Pathways

Betalains are composed of a betalamic acid moiety ($C_8H_9NO_5$) conjugated either with cyclo-DOPA (to form betacyanins) or amino acids/amines (to form betaxanthins). The conjugated imine bond between the aldehyde of betalamic acid and the amino group of cyclo-DOPA imparts vivid color but is also a key site of chemical vulnerability (Esteves et al., 2018). Degradation primarily involves oxidative cleavage of this imine bond or decarboxylation of carboxyl substituents under thermal and pH stress (Delgado-Vargas et al., 2000).

Thermal decomposition yields brownish products such as neobetanin and decarboxy-betanins. Light exposure accelerates photo-oxidation through singlet-oxygen attack on the betalamic chromophore, while metal ions like Fe^{3+} or Cu^{2+} catalyze oxidation reactions, leading to pigment precipitation and color loss. Oxygen availability amplifies these effects, especially in aqueous or high-moisture matrices.

In addition, betalains are hydrophilic molecules that dissolve readily in water and exhibit limited partitioning into lipid phases, which limits their stability in emulsions or lipid-rich systems (Stoica et al., 2025). Their zwitterionic nature renders them sensitive to ionic strength; electrolytes and salts can cause pigment aggregation or degradation via ionic interactions.

5.3 Influence of pH and Temperature

pH is a crucial factor that affects betalain stability. The best pigment stability occurs between pH 5 and 6, close to neutral, where the imine and carboxylic groups remain unprotonated and protected from hydrolysis (Delgado-Vargas et al., 2000). In acidic conditions (pH < 4), protonation of the imine nitrogen breaks the C=N bond, leading to color fading and the formation of colorless breakdown products. In alkaline conditions (pH > 8), deprotonation leads to nucleophilic attack on the betalamic ring, yielding yellowish derivatives (Kumorkiewicz-Jamro et al., 2021).

Temperature similarly affects betalain stability through thermally induced oxidation and decarboxylation. Betanin begins to degrade at temperatures above 60 °C, with half-life decreasing exponentially with temperature (Delgado-Vargas et al., 2000). Thermal stress also accelerates the conversion of betanin to neobetanin, which exhibits a duller hue. High-temperature processing, such as pasteurization or drying, results in marked pigment losses unless protective matrices or encapsulation are employed.

The combination of elevated temperature and low pH, as encountered in acidic beverages, can cause synergistic degradation. Kinetic studies reveal first-order degradation behavior with activation energies of 50-65 kJ mol^{-1} (Rodrigo et al., 2007). Thus, any process involving heat or pH manipulation must balance microbial safety with pigment preservation.

5.4 Effect of Oxygen, Light, and Metal Ions

Oxygen is a major contributor to betalain degradation through oxidative cleavage of the betalamic acid moiety. The presence of dissolved oxygen promotes radical formation, which converts betanin to betalamic acid and cyclo-DOPA-5-O-glucoside, thereby reducing chroma (Attoe & von Elbe, 2006). Reducing oxygen exposure during extraction and storage by vacuum packaging, nitrogen flushing, or adding antioxidants such as ascorbic acid significantly improves pigment stability.

Light, particularly ultraviolet radiation, induces photo-oxidation via single-oxygen mechanisms. Photodegradation produces intermediate radicals and rearrangement products that absorb at different wavelengths, manifesting as visible color shifts. Storing beetroot extracts in opaque or amber containers is a common strategy to mitigate photolytic damage (Ortmann et al., 2025).

Metal ions, including Fe^{3+}, Cu^{2+}, and $Mn^{2,+}$ catalyze redox cycling reactions, accelerating pigment oxidation (Chen et al., 2024; Nguyen et al., 2025). Chelating agents such as EDTA or citric acid are often incorporated into formulations to sequester metals and prevent catalytic degradation (Di Palma & Mecozzi, 2007). Deionized water and stainless-steel equipment are preferred during processing to minimize contamination from reactive metals (Silco Tek, 2021).

5.5 Influence of Matrix Composition and Water Activity

The stability of beetroot bioactives mainly depends on the surrounding matrix. In aqueous systems, betalains stay fully dissolved, making them more prone to oxidation and hydrolysis. Conversely, binding to polysaccharides or proteins can offer protection by decreasing molecular mobility and oxygen exposure (Abarca-Cabrera et al., 2021). For instance, adding beetroot extract into gum arabic or maltodextrin matrices during spray drying can improve pigment retention by up to 80% compared to free extracts.

Water activity (a_p) plays a dual role. Low water activity (< 0.3) stabilizes pigments by reducing mobility of reactive species, whereas intermediate a_p (0.5–0.7) can accelerate degradation due to optimal conditions for oxygen diffusion and pigment mobility (Stoica et al., 2025). High water activity (>0.8) again reduces degradation but may promote microbial spoilage, necessitating the use of additional preservatives. Consequently, controlling moisture content through drying, encapsulation, or the addition of humectants is essential for shelf-stable formulations.

5.6 Degradation During Processing and Storage

Processing methods such as juicing, pasteurization, freeze-drying, and encapsulation have varying effects on betalain stability, and Table 6 describes strategies for encapsulating and nanoformulating the beetroot bioactives. Mechanical disruption during juicing exposes pigments to oxygen and enzymes, initiating oxidative degradation. Pasteurization above 70 °C causes significant color loss, whereas mild pasteurization (≤ 60 °C, 30 min) maintains most betalain content while ensuring microbial safety (Delgado-Vargas et al., 2000).

Table 6. *Encapsulation and Nanoformulation Strategies for Beetroot Bioactives.*

Technique / Carrier	Particle Size (nm / µm)	Encapsulation Efficiency (%)	Advantages / Functions	Limitations
Spray-drying (maltodextrin + gum arabic)	5–50 µm	80–90	Scalable, cost-effective, good color stability	Thermal exposure may reduce antioxidants
Freeze-drying (carbohydrate matrices)	10–100 µm	85–95	Excellent preservation of thermolabile pigments	Slow, energy-intensive
Chitosan–TPP nanoparticles	100–300 nm	85–95	Electrostatic protection, pH-responsive release	Cost of polymer, aggregation risk
Liposomes / NLCs	50–200 nm	70–90	Enhance intestinal absorption, sustained release	Stability and storage challenges
Cyclodextrin inclusion complexes	Molecular (β-CD cavity)	60–80	Protect against oxidation, improve solubility	Limited loading capacity

Freeze-drying is one of the most effective preservation methods because sublimation at low temperature prevents oxidation and maintains pigment structure (Nowak & Jakubczyk, 2020). However, rehydration and subsequent storage at ambient temperature can still induce degradation if the product absorbs moisture.

During storage, temperature and packaging atmosphere strongly influence pigment half-life. Refrigerated storage (4–10°C) substantially slows degradation kinetics. For instance, betanin's half-life extends from 14 days at 25 °C to 56 days at 4 °C under dark, oxygen-limited conditions (Sadowska-Bartosz & Bartosz, 2021). Light exposure and high humidity accelerate color fading. These findings emphasize the need for cold-chain management and oxygen-barrier packaging in commercial beetroot products.

5.7 Interactions with Other Food Components

Beetroot bioactives interact with various food components, affecting stability and color expression. Sugars and organic acids stabilize betalains through hydrogen bonding and by decreasing water activity, while ascorbic acid offers antioxidant protection by neutralizing free radicals (Castro-Enríquez et al., 2020). However, a high amount of ascorbic acid can unexpectedly cause betalain breakdown under high oxygen levels due to pro-oxidant redox cycling. Proteins and polysaccharides can form weak complexes with betalains, enhancing thermal stability and preventing aggregation. Lipid interactions are limited since betalains are water-soluble, but emulsifiers and lecithin can help partition betalains at interfaces, improving color retention in emulsified systems. Polyphenols such as quercetin and catechins can act synergistically to protect betalains by neutralizing reactive oxygen species (Stoica et al., 2025). Understanding these beneficial and inhibitory interactions is essential for developing stable, functional foods and nutraceuticals from beetroot extracts (Table 7).

Table 7. Industrial and Nutraceutical Applications of Beetroot Bioactives.

Application Area	Product Type / Example	Functional Role of Beetroot Compounds	Industrial Benefit
Functional Foods	Dairy, bakery, beverages	Natural colorant, antioxidant fortification	Clean-label appeal, extended shelf life
Nutraceuticals	Capsules, gummies, drink powders	Cardiovascular and anti-inflammatory benefits	Added value through nitrate + betalain synergy
Cosmeceuticals	Antioxidant creams, gels	UV-protective and anti-aging activity	Natural pigment aesthetics, bio-safety
Pharmaceuticals	Encapsulated antioxidant formulations	Controlled release, oxidative stress therapy	Improved bioavailability and stability
Food Packaging / Biopolymer Films	Beetroot-pigment-infused coatings	Colorimetric freshness indicator, antioxidant agent	Intelligent / active packaging potential

6. Conclusion

Beetroot (*Beta vulgaris* L.) has gained remarkable scientific and industrial attention as a rich source of bioactive compounds, particularly betalains, phenolic acids, and flavonoids, with potent antioxidant, anti-inflammatory, and cardioprotective properties. However, their widespread use in functional foods and nutraceuticals remains limited by their intrinsic instability and poor bioavailability. Betalains are highly sensitive to environmental factors such as temperature, pH, light, and oxygen, which accelerate degradation, leading to color loss and reduced biological efficacy. Addressing these limitations requires both efficient extraction methods that preserve structural integrity and stabilization strategies that enhance shelf life and absorption.

Recent technological advances have transformed extraction science from conventional solvent-based approaches to green, energy-efficient techniques. Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) accelerate mass transfer by disrupting cell walls and volumetrically heating, thereby significantly increasing pigment yield and reducing solvent consumption. These techniques minimize exposure to high temperature and oxidation, preserving the antioxidant activity of beetroot compounds. In parallel, the development of deep eutectic solvents (DESs) has introduced a new generation of biodegradable, tunable, and non-toxic solvents capable of solubilizing and stabilizing betalains through strong hydrogen-bonding interactions. Integrating DESs with ultrasound or microwave energy further enhances extraction kinetics and pigment retention, aligning with the goals of sustainable bioprocessing and circular bioeconomy.

Post-extraction stabilization remains equally critical. Encapsulation and nanoformulation technologies have proven indispensable in overcoming degradation and bioavailability barriers. Techniques such as spray-drying and freeze-drying effectively produce microencapsulated beetroot powders with improved color stability, while lipid- and polymer-based nanocarriers—including liposomes, chitosan nanoparticles, and nanostructured lipid carriers—provide controlled release and protect bioactives during gastrointestinal digestion. These systems not only prevent oxidation and photolysis but also enhance solubility and intestinal permeability, thereby increasing bioaccessibility and improving efficacy. Emerging hybrid systems combining green extraction and encapsulation, such as ultrasound-assisted or DES-based encapsulation, represent a significant step toward process intensification and industrial scalability.

Despite this progress, key challenges persist. The standardization of extraction parameters, validation of solvent and carrier safety, and scaling-up of nanotechnologies remain priorities before widespread commercialization can be achieved. Furthermore, a deeper mechanistic understanding of pigment–matrix interactions, degradation kinetics, and absorption pathways must be developed through *in vitro* digestion and *in vivo* pharmacokinetic studies. Interdisciplinary collaboration among food scientists, process engineers, and materials chemists will be crucial to create reproducible, economically feasible, and regulatory-compliant solutions.

In conclusion, advances in green extraction, encapsulation, and nanodelivery have transformed beetroot from a simple natural pigment source into a sustainable platform for high-value nutraceuticals and functional ingredients. The integration of green chemistry, material science, and food engineering now enables the production of stable, bioavailable, and environmentally responsible beetroot-derived compounds. Continued innovation in solvent design, nanoencapsulation, and biorefinery integration will further unlock the full potential of beetroot as a model for sustainable bioactive recovery and functional food development.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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