

Systematic Review

Deep Eutectic Solvents (DES) and Natural Deep Eutectic Solvents (NADES)-Mediated Extraction of Bioactives from Brewing By-Products: A Scoping Review for Functional Food and Sports Supplementation

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Abstract

The brewing industry generates substantial organic waste, prompting interest in sustainable valorization because these by-products retain significant residual nutritional and nutraceutical value. Based on these premises, this scoping review aims to evaluate whether DES- and NADES-mediated extractions can represent a green alternative to conventional organic solvents, assessing their capacity to yield stable, biocompatible extracts with potential nutritional relevance for athletic supplementation. Following PRISMA-ScR guidelines and prospectively registered on OSF, we screened PubMed, Scopus, and Web of Science databases in June 2026, including 22 primary studies. Results show that DES and NADES can effectively dismantle brewing by-products, recovering antioxidants—specifically ferulic acid, p-coumaric acid, and xanthohumol (up to 285.92 mg GAE/100 g dw)—protein concentrates (up to 54.7 wt% total protein) rich in essential amino acids and branched-chain amino acids (BCAAs), as well as immunomodulatory prebiotic fibers (β -glucans, arabinoxylans) and liquid phases that bio-ferment into polyunsaturated fatty acids (PUFAs). Non-conventional technologies such as ultrasound and microwave-assisted extraction accelerate extraction kinetics by reducing solvent viscosity. It should be emphasized that the included studies are exclusively in vitro and characterize extracted compounds without evaluating effects on human subjects or athletic performance. However, several safety and regulatory gaps remain, including the presence of gluten in barley-derived extracts, the potential co-ingestion of antinutritional factors (e.g., oxalic acid), and the lack of toxicological data on intact eutectic phases. While DES- and NADES-derived extracts show promising in vitro bioactivities, further research is needed to validate their safety, bioavailability, and efficacy before they can be considered for human consumption or sports nutrition applications.

Keywords: green extraction; biosustainability; brewers' spent grain (BSG); brewers' spent hops (BSH); sport nutrition and supplementation; functional foods; polyphenols; proteins; nutraceuticals; pharmaceuticals



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1. Introduction

The brewing industry ranks among the most significant segments of the global agri-food sector, while concurrently generating substantial volumes of organic effluents and residues. Currently, beer remains the predominant alcoholic beverage in terms of global consumption, with annual volumes hovering around 200 billion liters [1]. The brewing by-products originating from this supply chain are primarily concentrated into a series of macroscopic fractions analyzed within the scope of this work: brewers' spent grain (BSG), brewers' spent hops (BSH), brewers' spent yeast (BSY), malt rootlets (MR), and malt dust (MD) [2,3]. Although some of these biomasses have historically played a secondary role due to their lower volumes compared to the main byproduct, this study systematically inventories and analyzes them to map their latent bioactive potential.

BSG represents the most quantitatively significant solid fraction of the entire process, accounting for approximately 85% of the total by-products generated during brewing; specifically, the production of 100 L of beer yields a recovery of about 20 kg of BSG [4]. From a structural standpoint, BSG constitutes the insoluble portion of the barley grain remaining after the mashing and filtration (lautering) stages, retaining both residual soluble components (glucose (Glc), fructose (Fru), maltose, and maltooligomers) and structural macromolecules such as fiber, proteins, pectins, and complex lignocellulosic polysaccharides—as well as polyphenolic antioxidants, functional lipids, and bioactive secondary metabolites [5–9]. To date, traditional management routes are mainly divided between the animal nutrition sector (70%) and energy conversion via biogas production (20%), while the remaining share (10%) is discarded in landfills without any form of valorization [10–12].

Beyond animal nutrition and sporadic applications in human nutrition [13,14], the brewing by-products are suitable for heterogeneous technological solutions: from energy conversion via pyrolysis for the production of bio-oil, bio-char, and gases [15], to paper manufacturing or the use of biomass as a bio-adsorbent for treating industrial effluents and gaseous emissions [16]. The emergence of circular economy paradigms and innovations in biotechnological processes have catalyzed interest in advanced valorization strategies.

To fully exploit the bioactive potential of these brewing by-products, conventional extraction methods relying on volatile organic solvents face significant limitations regarding toxicity, environmental impact, and industrial sustainability [17,18]. To overcome these barriers, a paradigm shift inspired by green chemistry focused initially on the development of Deep Eutectic Solvents (DES) [19,20]. From a structural standpoint, a DES is formed by a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) that self-associate at a specific molar ratio [21,22]. The primary mechanism driving these solvents is a dense network of intermolecular hydrogen bonds; this interaction induces charge delocalization and disrupts the crystalline lattice of the individual components, causing a drastic depression of the melting point [23,24]. A classic example is reline, where choline chloride (ChCl) and urea (U) are mixed in a 1:2 ratio to yield a room-temperature liquid with a melting point of just 12 °C [25]. For industrial or laboratory applications, DES can be synthesized through various techniques—most commonly heating and stirring, but also via microwave, ultrasonication, grinding, freeze-drying, or vacuum evaporation [26,27]. Based on their chemical makeup, these multi-component systems are traditionally classified into four categories (Types I to IV) depending on the nature of the components involved [28].

Intriguingly, metabolomic investigations later revealed that nature had already engineered a similar phenomenon within living organisms. This led to the conceptualization of Natural Deep Eutectic Solvents (NADES), based on the discovery that cells naturally synthesize a “third liquid phase”—completely distinct from water and lipids—composed exclusively of ubiquitous primary metabolites [19]. Depending on the hydrogen-bond

donor selected, they form a highly tunable platform classified into sugar, amino acid, organic acid, polyol, or hydrophobic terpene-based systems [22,29,30]. Chemically, their dense hydrogen-bonding network often results in high viscosity, a limitation easily managed via mild heating or controlled water addition to optimize mass transfer [31,32]. Furthermore, their non-volatile nature eliminates the need for subsequent solvent removal steps [32], allowing specific formulations—such as ChCl mixed with organic acids—to effectively solubilize and stabilize target bioactives under mild processing conditions [33].

The extraction of bioactive compounds from brewing by-products using DES and NADES represents a pivotal opportunity to develop ingredients for the manufacturing of functional foods and dietary supplements. This approach aligns with modern clinical and public health paradigms, which increasingly recognize the manipulation of dietary factors as a therapeutic strategy to preserve health across the lifespan [34,35]. In this context, regular physical activity is globally recommended for its health benefits [36–40]. However, intense or prolonged exercise triggers overproduction of reactive oxygen species (ROS) [41–43] and muscle catabolism [44–46]. To mitigate these effects and support recovery, supplementation with exogenous antioxidants [47,48], amino acids and peptides [49,50], and anti-inflammatory lipids [51,52] has become a common practice.

Based on these premises, this scoping review addresses the research question of whether DES- and NADES-mediated extractions can serve as a viable green alternative to conventional organic solvents for yielding stable, biocompatible extracts with potential nutritional value for athletic supplementation. To answer this question, this work maps the current landscape of brewing by-product valorization. The primary objective is to evaluate the extraction yields, antioxidant capacities, and specific polyphenol and protein profiles obtained from brewing by-products when utilizing DES and NADES. As a secondary objective, this study aims to assess the biocompatibility, safety, and nutritional relevance of the resulting extracts, based on their characterized *in vitro* profiles, and to discuss their potential use as dietary supplements to counteract oxidative stress, support muscle recovery, and support athletic performance.

2. Materials and Methods

2.1. Study Design and Registration

This methodological and technological scoping review was planned and executed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 extension for Scoping Reviews (PRISMA-ScR) guidelines [53]. To ensure methodological transparency and prevent selection bias, the study protocol was prospectively registered on the Open Science Framework (OSF) [54] on 9 June 2026 and updated on 4 August 2026 to reflect the scoping review framework (Registration DOI: <https://doi.org/10.17605/OSF.IO/ZEV8W>). In accordance with the PRISMA-ScR methodology, this scoping review aims to map the available evidence and address specific research questions, as the included primary literature focuses on laboratory-based experimental studies without human subjects or animal models.

2.2. Eligibility Criteria

The criteria for study eligibility were established *a priori* to define the precise technical boundaries of the literature screening. Because this review addresses a methodological and technological question rather than a clinical one, the evaluation framework was based on specific laboratory and chemical domains. Regarding the target matrices, inclusion was strictly restricted to studies utilizing agro-food by-products or brewing by-products, specifically focusing on BSG, BSY, and BSH. Consequently, investigations evaluating biomasses

from other agri-food sectors, such as winemaking, juice processing, or agricultural crop residues unrelated to brewing, were excluded.

In terms of the extraction agents, the eligibility scope encompassed original research employing either DES or NADES as the primary media for isolating bioactive fractions. This inclusive paradigm was maintained to systematically map the baseline extraction efficiencies of eutectic mixtures against traditional methods; however, the biocompatibility and subsequent food-grade or functional relevance of these formulations were critically evaluated against sports nutrition requirements based on the *in vitro* and chemical data extracted from the included studies, differentiating non-toxic natural combinations from potentially toxic synthetic variants. Studies that relied solely on conventional, petroleum-derived organic solvents—such as methanol, ethanol, or acetone—or water without a direct comparison to eutectic systems were deemed ineligible.

Furthermore, included studies were required to report quantitative data on specific target outcomes, including the extraction yield or recovery efficiency of total phenolic content, individual polyphenolic profiles characterized via chromatographic or spectrophotometric techniques, or total protein and amino acid yields. Eligible papers were also required to provide quantitative values for antioxidant capacity, evaluated through standard assays such as DPPH, ABTS, or FRAP. Finally, the selection was limited to original, peer-reviewed research articles containing primary experimental data, thereby excluding document typologies, such as narrative reviews, scoping reviews, book chapters, conference abstracts, patents, and editorials. To ensure a standardized synthesis, the eligibility framework was restricted to articles published exclusively in the English language.

2.3. Information Sources and Search Strategy

To identify relevant primary research articles, a comprehensive and systematic literature search was performed across three electronic databases: PubMed/MEDLINE, Scopus, and Web of Science. These specific platforms were selected to ensure a complementary and exhaustive coverage of biomedical, chemical, engineering, and interdisciplinary health science literature. The electronic search was executed in June 2026, and no *a priori* chronological restrictions or filters were applied to the publication dates, thereby capturing all historical literature up to the search date.

The search strings were constructed using a combination of specific keywords tailored to the syntax and field requirements of each database, focusing on the target brewing by-products and eutectic extraction systems. For the PubMed database, the query was executed using the following string:

“brewers spent grain” OR “brewer’s spent grain” OR “brewers spent grains” OR BSG OR “malt dust” OR “spent hops” OR “spent yeast”) AND (“deep eutectic” OR NADES OR “natural deep eutectic”).

This strategy was adapted for Scopus by applying the search terms within the title, abstract, and keyword fields through the following configuration:

TITLE-ABS-KEY (“brewers spent grain” OR “brewer’s spent grain” OR “brewers spent grains” OR BSG OR “malt dust” OR “spent hops” OR “spent yeast”) AND TITLE-ABS-KEY (“deep eutectic” OR NADES OR “natural deep eutectic”).

For the Web of Science Core Collection, the search was conducted within the Topic (TS) field using the identical keyword combination:

TS = (“brewers spent grain” OR “brewer’s spent grain” OR “brewers spent grains” OR BSG OR “malt dust” OR “spent hops” OR “spent yeast”) AND TS = (“deep eutectic” OR NADES OR “natural deep eutectic”).

In addition to these electronic database queries, a manual screening of the reference lists of all retrieved narrative reviews, systematic reviews, and meta-analyses was con-

ducted to identify any further primary experimental studies that the initial electronic search strategy might have omitted.

2.4. Selection Process

Following the electronic database searches, all retrieved bibliographic records were exported and consolidated into Zotero reference management software (version 9.0.5, 64-bit, Corporation for Digital Scholarship, Vienna, VA, USA). Internal automated duplication filters were applied within the software to systematically identify and remove exact duplicate entries, which were subsequently cross-verified through a manual sweep to ensure no distinct primary records were erroneously deleted.

The standardized screening process was then executed across two distinct, consecutive phases by two researchers working independently to minimize selection bias. In the first phase, the titles and abstracts of all non-duplicate records were evaluated against the predefined eligibility criteria. Articles that clearly focused on non-brewing matrices, conventional solvent systems without eutectic comparison, or non-experimental document types were discarded at this stage.

Records that successfully passed the initial screening, or those with insufficient information in the abstract to warrant immediate exclusion, progressed to the second phase, which involved a meticulous examination of the full-text documents.

During both the title/abstract and full-text screening stages, any discrepancies or disagreements between the two primary researchers regarding the inclusion or exclusion of a specific study were resolved through open technical discussion until a consensus was reached; if a disagreement had persisted, a third senior researcher was available to arbitrate and make the final determination. Neither of the screening researchers was blind to the authors, institutions, or journal names of the publication records under evaluation.

2.5. Data Collection Process

A dedicated and standardized data extraction form was developed in Microsoft Excel to ensure consistency and systematic acquisition of information from the final pool of included primary studies. The extraction process was carried out independently by two researchers, who carefully audited each full-text article to populate the database.

Any conflicting interpretations of the extracted technological or chemical variables were resolved through direct consultation and technical discussion between the two extractors until consensus was reached.

To ensure the integrity and accuracy of the final dataset, the completed extraction sheets were cross-checked and integrated, verifying that all parameters reflected the exact experimental configurations described in the primary literature.

2.6. Data Items

The data extraction process was structured to methodically map every variable required to evaluate the nutraceutical potential of the investigated matrices, strictly mirroring the sequential architecture of the scoping review's master table. Initially, the included studies were mapped and organized chronologically within Table 1, dedicating specific entries for the identification of each article by indicating its author and publication date.

The first core set of extracted data defined the nature of the agri-food by-products, which mainly originate from brewing industry waste (e.g., BSG, BSH). Together with the matrix type, the specific physical or chemical pre-treatments applied before extraction were recorded, including drying temperatures and milling mesh sizes to control particle granulation.

Regarding the extraction solvents, the collected data captured their molecular formulation and any water dilution percentages. To evaluate the green profile and selectivity of

the media, the mixtures were systematically categorized based on the nature of their components. Specifically, they were classified as NADES when both the HBA and HBD were metabolites of entirely natural origin, while systems incorporating synthetic or industrial reagents were categorized as DES.

The investigation then gathered all operational and engineering variables governing the extraction process. This included the specific solid-to-liquid ratio, extraction temperature, processing time, and the technological configuration utilized, explicitly differentiating among conventional heated maceration, microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE). Fabricating a clear picture of these items, along with downstream separation and purification conditions like centrifugation speed, filtration methods, or membrane dialysis, is essential to correlate the energy input and mechanical shear with the overall efficiency of macromolecular release.

Finally, the target outcomes were extracted and partitioned to comprehensively evaluate the functional and nutritional valorization of the resulting streams. Phenolic recovery values were recorded as total phenolic content or via the absolute quantification of individual monomeric phytochemicals cleaved from the matrix, such as ferulic acid, p-coumaric acid, or catechol. Concurrently, data concerning the capacity to capture free radicals were extracted based on specific biochemical assays to evaluate how processing parameters influence the preservation of thermolabile structures. Under this same outcome domain, other macromolecular and biological variables were gathered, including protein yields, structural carbohydrate profiles, and downstream bioconversion metrics like fungal mycelial colonization or organic acid production in secondary fermentations, ensuring a complete assessment of the functionalized extracts.

For any missing, incomplete, or unclear technical information or operational parameters within the primary studies, no assumptions were made, and the data were treated as not reported.

2.7. Synthesis Methods

A qualitative and narrative synthesis approach was adopted to group and contrast the findings from the included studies, as the heterogeneity in raw material origins, extraction technologies, and operational parameters precluded a formal quantitative meta-analysis. To complement the narrative synthesis, a frequency analysis of the solvent systems and extraction techniques was conducted. The results are presented as a heatmap and descriptive summary.

The analytical data gathered from the extraction processes and chemical outcomes were tabulated and clustered to be comprehensively synthesized and interpreted within Section 4 through a three-step progressive evaluation.

First, the synthesis focuses on critical considerations regarding the matrices, evaluating how the inherent variability of brewing by-products and their respective stabilizing pre-treatments influence the accessibility of target molecules.

Second, general considerations regarding the bioactive outcomes are addressed; here, data are compared based on the chemical classification of the eutectic and stratified by extraction technology (conventional, microwave, or ultrasound) to identify the most efficient systems for total phenolic content recovery and macromolecular extraction.

Finally, these synthesized findings are translated into a targeted evaluation focused on sports nutrition and athletic supplementation. In this final stage, the extracted bioactives and co-extracted nutrients are discussed in relation to their functional potential to counter exercise-induced oxidative stress, support muscle metabolic recovery, and provide natural, bio-based ingredients suitable for performance-supplementation formulations, based on the *in vitro* profiles characterized in the included studies. Where applicable, extraction

yields are benchmarked against dietary reference intakes and supplementation guidelines from the International Society of Sports Nutrition (ISSN). This benchmarking is intended strictly as a quantitative evaluation of the raw material's bioactive capacity—quantifying the theoretical payload present in the waste stream—rather than a direct indication of finished supplement dosage or clinical efficacy.

3. Results

3.1. Study Selection

The scoping literature search across PubMed, Scopus, and Web of Science yielded 75 records. After removing 34 duplicate entries, 41 unique records were screened based on title and abstract, leading to the exclusion of 7 articles.

The full texts of the remaining 34 reports were successfully retrieved ($n = 0$ unavailable) and evaluated for eligibility. Of these, 12 reports were excluded for the following reasons: document not available in English but in Chinese ($n = 1$), non-experimental study design such as reviews or editorials ($n = 8$), and off-topic scope without eutectic solvents, brewing matrices, or focus on health-beneficial properties ($n = 3$).

Ultimately, 22 original research articles met all eligibility criteria and were included in the qualitative synthesis. The complete screening process and the exact flow of records are visually summarized in the PRISMA 2020 flow diagram (Figure 1; Supplementary File S1).

3.2. Study Characteristics and Key Bioactive Outcomes

The general architectural, chemical, and operational features of the 22 included studies reflect a highly heterogeneous body of literature published predominantly over the last decade, highlighting the recent and growing academic interest in green extraction technologies applied to agri-food co-products. In terms of raw materials, the vast majority of the investigated literature focuses on BSG, followed by minor but highly specialized representations of BSH and malt sprouts (MR and MD). Regarding the extraction media, a clear trend emerges toward the use of NADES over DES. ChCl represents the overwhelmingly dominant HBA utilized across the literature, with betaine (Bet) appearing as the primary bio-based alternative. In terms of HBD, polyols—predominantly glycerol (Gly) and ethylene glycol (EG)—and organic acids, such as lactic acid (LA), malic acid (MA), and citric acid (CA), are the most frequently investigated, alongside emerging alkaline or acid-catalyzed mixtures designed to maximize matrix deconstruction. Analyses of the principal bioactive outcomes reveal three main trends. First, a substantial recovery of polyphenols (primarily ferulic acid, p-coumaric acid, catechin, catechol, and syringaldehyde) with high radical scavenging activity, anti-neurodegenerative potency (e.g., α -synuclein fibril inhibition), and the capacity to downregulate pro-inflammatory genes—interleukin-6 (IL-6) and interleukin-1 β (IL-1 β)—in cell models, alongside the selective isolation of prenylflavonoids like xanthohumol from hop matrices. Second, an efficient valorization of macronutrients, yielding protein-rich dry concentrates (containing up to 54.7 wt% total protein with extraction yields reaching 79%) characterized by balanced essential amino acid profiles, as well as carbohydrate fractions with prebiotic potential (glucans and arabinoxylans). Third, the innovative upcycling of the liquid hydrothermal phases as chemical-free nutrient growth media for microbial bio-fermentations to produce astaxanthin, PUFAs, and branched-chain fatty acids (BCFAs). A comprehensive overview of these outcomes for each included study is presented in Table 1.

Table 1. Characteristics of studies included.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Allegretti et al., 2022 [55]	BSG Dried (60 °C, 24 h) and ground. Autoclaved (121 °C, 15 min).	ChCl:LA (1:5) (NADES)	Hydrothermal hydrolysis and microbial fermentation (5 L bioreactor). Downstream: 22–28 °C, pH 6.5–7.0, stirring at 200 rpm, aeration at 0.6 L/L/min for 4–6 days.	Astaxanthin-rich Biomass (<i>P. rhodozyma</i>): Yield up to 3.6 g/L (135 g/kg BSG). PUFAs/Linoleic acid (<i>Y. lipolytica</i>): 72% of the total lipid fraction (biomass yield: 1.5–1.6 g/L; lipid yield: 200–220 mg/L). BCFAs (<i>Streptomyces</i>): High concentration of branched-chain fatty acids (biomass yield: 1.7–2.1 g/L; lipid yield: 180–220 mg/L). Total Fatty Acids (<i>R. opacus</i>): Biomass yield up to 2.4 g/L; lipid yield up to 547 mg/L.
Antoun et al., 2024 [56]	BSG Pressed (2 bar, 5 min), dried (75 °C, 48 h), and ball-milled (5 min, 30 Hz).	ChCl:LA (1:2) ChCl:OA (1:2) ChCl:FA (1:2) (NADES)	MAE (2.45 GHz, 600 W). 4 g BSG in 20 mL NADES. Tested: 130–170 °C for 15 min/150 °C for 10–30 min. Downstream: Ethanol (1:2), water precipitation (1:3), centrifugation (4 °C, 5000 rpm, 20 min).	Antioxidant lignin: Yield up to 16.65% (166.5 g/kg BSG; representing up to 99.64% of the initial lignin). High purity (74.03–87.96% Klason lignin). Highly efficient radical scavenging activity (IC ₅₀ : 0.022–0.040 mg/mL). Bioactive proteins: Significant residual co-fraction rich in essential amino acids (phenylalanine and tyrosine). Residual carbohydrates and lipids: Co-isolated fraction containing hemicelluloses (<0.5 wt%) and trace lipids (triglycerides).
Bleus et al., 2024 [57]	BSG and MDDried (65 °C, residual water content ≤5 wt%).	ChCl:MA (1:1) ChCl:Gly (1:2) (NADES)	Processes tested separately (1:20 <i>w/w</i> biomass-to-solvent ratio): Heated and stirred maceration: 90 °C (70 °C for acetone), 60 min, 500 rpm. Hydrothermal extraction: 120 °C, 60 min, 260 rpm (pressures: 0.8 bar for NADES, 4.6 bar for acetone). MAE: 90 °C, 15 min isothermal, magnetic stirring (40% intensity). Downstream: Centrifugation (9000 rpm, 5 min, repeated twice).	Ferulic acid: via hydrothermal extraction with malic acid: 272 µg/g dw for BSG and 52 µg/g dw for MD. P-coumaric acid: via hydrothermal extraction with MA: 92 µg/g dw for BSG and 17.2 µg/g dw for MD. Caffeic acid: via maceration with Gly: 21.2 µg/g dw for BSG and 20.5 µg/g dw for MD. Syringaldehyde: via hydrothermal extraction with MA: 375 µg/g dw for BSG and 103 µg/g dw for MD (max cumulative yield). Matrix breakdown: hemicellulose and lignin degradation verified via 13C-NMR and py-GC-MS during hydrothermal extraction with MA.
Cassoni et al., 2023 [58]	BSG Dried till constant weight and milled (particle size ranging from 800 to 2000 µm).	ChCl:LA (1:5) (NADES)	Process tested (1:10 <i>w/w</i> biomass-to-solvent ratio): heated and stirred extraction, tested at 60, 90, and 120 °C for 1, 3, and 5 h. Downstream (lignin recovery): filtration, precipitation with deionized water (3:1 <i>v/v</i> at 4 °C overnight), centrifugation (5000 rpm, 20 min), washed two times with deionized water (5000 rpm, 15 min), and oven-drying at 100 °C.	Lignin yield: 54.4% for BSG (at 120 °C for 5 h). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) scavenging activity: 682.1 µmol Trolox Equivalents/g lignin for BSG. 2,2-diphenyl-1-picrylhydrazyl scavenging activity: 872.1 µmol Trolox Equivalents/g lignin for BSG. Total phenolic content: 88.8 mg GAE/g lignin for BSG. Extract characterisation: Purity of the recovered fraction verified at 77.2% with a molecular weight of 27,591 g/mol and a polydispersity index of 1.34.

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Grudniewska et al., 2020 [59]	BSH Sieved through a 1 mm sieve.	ChCl:Gly (1:2) ChCl:EG (1:2) ChCl:LA (1:2) (NADES) ChCl:PG (1:2) (DES)	Heated and stirred extraction, tested at 40, 50, 60, 70, and 80 °C for 15, 30, 60, and 120 min (biomass-to-solvent ratios from 1:10 to 1:50 <i>w/w</i>). Control extraction performed with methanol or acetone at room temperature for 1 h. Downstream (xanthohumol recovery): extrusion pressing, water-induced precipitation (3:1 to 5:1 <i>v/w</i> at 4 °C for 12 h), centrifugation (4075× <i>g</i> , 10 min, 4 °C), and final dissolution in methanol.	Initial xanthohumol yield (1:10 <i>w/w</i>): 0.42 mg/g (ChCl:Gly), 0.68 mg/g (ChCl:EG), 0.75 mg/g (ChCl:LA), 0.75 mg/g (ChCl:Pg). Optimized maximum xanthohumol yield: 2.30 mg/g (with ChCl:Pg at 1:50 <i>w/w</i>).
Grudniewska et al., 2023 [60]	BSH Sieved through a 1 mm sieve.	ChCl:Gly (1:2) ChCl:LA (1:2) (NADES) ChCl:EG (1:2) ChCl:PG (1:2) (DES)	Heated and stirred extraction at 60 °C for 1 h. Downstream (xanthohumol recovery): extrusion press filtration (200-µm), centrifugation (4075× <i>g</i> , 30 min), precipitation with deionized water (4 °C, 12 h), centrifugation (3220× <i>g</i> , 30 min), washing (3 times), freeze-drying, and back-extraction with methanol.	Xanthohumol yield: Ranging from 0.52 mg/g BSH (with ChCl:Gly) to 1.92 mg/g BSH (with ChCl:LA).
Hadinoto et al., 2025 [61]	BSG Lyophilized and thawed at room temperature for 3 h.	ChCl:Tre (3:1) (NADES)	Heated and stirred extraction in a shaking incubator for 1.5 h. Control process performed at 60 °C with a 1:15 <i>w/v</i> ratio. Downstream (Protein recovery): centrifugation (1500× <i>g</i> , 20 min), precipitation with excess deionized water (15:1 <i>v/v</i>), centrifugation (5000× <i>g</i> , 10 min), water-washing (twice), and freeze-drying (−52 °C, 0.05 mbar, 24 h).	Protein yield: 42.0 ± 0.7 wt%. Essential amino acids profile: 41.98 ± 2.10 wt% of the total amino acid content. The most abundant are L-Lysine (8.49%), L-Leucine (7.19%), L-Phenylalanine (7.15%), L-Threonine (5.76%), L-Isoleucine (4.59%), and L-Valine (4.14%).

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Hernández-Corroto et al., 2023 [62]	BSG and MR Dried, ground, and stored at -20°C .	ChCl:Glc (2:1) ChCl:Glc (1:1) ChCl:Suc (4:1) ChCl:Gly (1:1) ChCl:CA (1:1) ChCl:CA (2:1) Bet:U (1:2) Bet:Gly (1:1) Bet:EG (1:2) Bet:Glc (1:3) (NADES) ChCl:EG (1:2) ChCl:U (1:2) ChCl:AA (1:2) GuCl:U (1:2) (DES)	HIFU extraction. Screening: 30 mg sample in 5 mL solvent, 1 min, 30% amplitude, room temperature. Optimization (Box–Behnken): sample amount (20–130 mg), HIFU amplitude (20–80%), temperature ($25\text{--}80^{\circ}\text{C}$), and time (1–20 min). Downstream: centrifugation ($4000\times g$, 10 min). Purification tested via precipitation (ammonium acetate in MeOH 1:3 or 35% TCA 1:1, 4°C overnight), SPE (C18, Strata-X, or Strata-X-CW columns eluted with 5% formic acid in MeOH), or 3 kDa membrane ultrafiltration.	Protein yield: Optimal recovery of 13 g protein/100 g for BSG and 10.2 g protein/100 g for MR. Bioactive peptide profile: Identification of 95 peptides in BSG and 87 peptides in MR via ex novo sequencing, including the specific sequence VIEPR. Co-extracted bioactives and antioxidant capacity: total phenolic content/antioxidant capacity (via Folin–Ciocalteu) was 22.7 ± 1.8 mg GAE/g for BSG and 14.1 ± 0.5 mg GAE/g for MR. HPLC-MS/MS identified the antioxidant ferulic acid in BSG, with no phenolic compounds detected in MR.
López-Linares et al., 2021 [63]	BSG Washed, oven-dried at 50°C , milled to a particle size lower than 1 mm, and homogenized (2% residual moisture content).	ChCl:LA (1:2) ChCl:Gly (1:2) (NADES) ChCl:EG (1:2) ChCl:Tre (1:2) (DES)	MAE and screening at 10% <i>w/v</i> ratio (5 g dry BSG in 50 mL DES), 65°C , 20 min, max power 700 W. Downstream: Cooling to 50°C , vacuum filtration, and solid phase washing with distilled water followed by drying at 40°C .	Total Phenols: 2.89 mg GAE/g BSG under optimized conditions (100°C , 13.30 min, 37.46% water in ChCl:Gly). Antioxidant Profile: High-purity radical scavengers dominated by Ferulic acid (26.4%) and Coumaric acid (20.6%), 4-hydroxybenzoic acid (14.4%) and Syringaldehyde (14.0%). Prebiotic Solid Fraction: 94.1% glucan and 97.7% hemicellulose retention in the solid residue, yielding 18.86% cellulose and 24.31% hemicellulose for prebiotic arabinoxylans upcycling.

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Masiello et al., 2026 [64]	BSG Oven-dried at 55 °C for 72 h, milled to fine powder, and sieved (median particle diameter D50 of 357.47 µm).	ChCl:LA (1:2) ChCl:Gly (1:2) ChCl:Glc (1:2) ChCl:Fru (1:2) Bet:LA (1:2) Bet:Gly (1:2) Bet:Glc (1:2) Bet:Fru (1:2) (NADES)	Maceration on a heating plate. Process parameters: solid-to-liquid ratio of 1:10 g/mL, incubated at 50 °C for 1 h under continuous magnetic stirring (200 rpm) in the dark. Downstream: Centrifugation at 3000 rpm for 15 min, followed by supernatant filtration through 0.45 µm membrane filters.	Total Phenols: ChCl:LA (285.92 mg GAE/100 g dw) and Bet:Gly (283.55 mg GAE/100 g dw) achieved the highest yields. Antioxidant Profile: Highest radical scavenging capacity found in ChCl:LA (85.54 µM TE/100 g dw in DPPH) and Bet:LA (92.2 µM TE/100 g dw in ABTS). Chromatographic analysis confirmed a substantial recovery of ferulic acid (up to 93.00 mg/100 g in Bet:Gly) and p-coumaric acid (up to 54.45 mg/100 g in ChCl:LA), alongside Catechin (9.12 mg/100 g), Syringic acid (3.38 mg/100 g), and Sinapic acid (2.08 mg/100 g).
Mikulski et al., 2025 [65]	BSG Ground via hammer mill (92.7 ± 0.1% DW).	ChCl:Gly (1:1, 1:2, 1:4) ChCl:U (1:1, 1:2, 1:4) ChCl:S (1:1, 1:2, 1:4) ChCl:Imi (1:1, 1:2, 1:4) (NADES) ChCl:EG (1:1, 1:2, 1:4) ChCl:Gly/U/S/Imi/EG + 0.9% v/v H ₂ SO ₄ (DES)	MAE (600 W, 60 min, 0.27/0.54 MPa) at 1:10 solid-to-liquid ratio (3 g DW BSG + 30 g solvent) in Teflon vessels (HP-500 Plus). Downstream: Cooled to 50 °C, filtered under lowered pressure, rinsed with 2000 mL distilled water, and dried at 70 °C for 8 h.	Phenolic Extraction Yield: Maximum recovery of the depolymerized phenolic fraction driven by a 77% cleavage of the BSG lignin matrix using ChCl:Imi (1:4 ratio, 0.54 MPa). Aromatic Ring Cleavage: High-efficiency degradation of complex lignin into low-molecular-weight phenolics, confirmed by the reduction in aromatic FTIR peaks at 1515 and 1660 cm ⁻¹ (ChCl:Imi and acid-catalyzed ChCl:Gly). Bioactive Liberation: Complete disruption of cell wall ester/ether linkages via optimized NADES/DES (ChCl:Imi and ChCl:EG), forcing the migration of bound antioxidant components into the liquid extract.
Mikulski et al., 2026 [66]	BSG Rinsed with 70 °C water until the extract reached 0.2 ± 0.1% w/w. Dried at 60 °C for 8 h to a dry weight of 92.7 ± 0.1%, then ground using a hammer mill to particles smaller than 2 mm.	ChCl:Gly (1:4) ChCl:Imi (1:4) (NADES) ChCl:Gly (1:4) + 0.9% w/w H ₂ SO ₄ ChCl:EG (1:4) + 0.9% w/w H ₂ SO ₄ (DES)	MAE pretreatment (600 W, 60 min, 39/78 psi) at 1:10 solid-to-liquid ratio (4 g DW BSG + 40 g solvent) in Teflon vessels. Downstream: Cooled to 40 °C, filtered, washed with 300 mL water, and dried at 70 °C for 8 h. Recirculation (5 cycles for ChCl:Imi at 78 psi): Vacuum filtration, centrifugation at 8000× g (20 °C), and concentration of the supernatant under reduced pressure.	1,2-dihydroxybenzene (catechol): Identified as the main bioactive compound from lignin depolymerization. Extraction reached ca. 80 mg/L with ChCl:Imi at 78 psi (with zero sugar dehydration by-products), and peaked at 278.6 mg/L when using the acid-catalyzed mixture (ChCl:EG + 0.9% H ₂ SO ₄). Other Bioactive Phenolics: Liberation of minor antioxidant and phenolic compounds from the BSG matrix, specifically including vanillin, p-coumaric acid, trans-ferulic acid, and coniferyl alcohol.

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Morán-Aguilar et al., 2022 [67]	BSG Crushed with a stainless-steel mill.	ChCl:U (1:2) ChCl:Gly (1:2) (NADES)	1:16 solid-to-liquid ratio, 15 h, 140 °C for ChCl:U/160 °C for ChCl:Gly, under magnetic stirring in a sand bath. Downstream: Quenched with 50 mL distilled water, centrifuged at 2755× g for 30 min, and CRM sediment dried at 50 °C for 24 h.	Selective Lignin Fractionation: ChCl:Gly extracted 79.27 ± 4.35% of acid-soluble lignin but left the heavy Klason lignin in the solid, migrating only small bioaccessible phenolics into the liquid phase. Viscosity-Limited Dissolution: ChCl:U achieved lower phenolic extraction (54.63 ± 1.12% acid-soluble lignin removal) due to weak alkalinity (pH 9) and high viscosity. Cell Wall Shielding Collapse: Total arabinan removal (0.00% left) via ChCl:Gly, dismantling the hemicellulose-lignin network that traps the bioactives.
Morán-Aguilar et al., 2025 [68]	BSG Liquid-to-solid ratio of 15:1 (v/w) at 130 °C for 90 min in a sand bath.	ChCl:Gly (1:2) ChCl:LA (1:4, 1:8, 1:12) (NADES)	Thermal processing: LSR 15:1 v/w, 130 °C, 90 min. Downstream: Quenched with acetone-distilled water (1:1 v/v) at an LSR of 25:1 (mL:g), orbitally stirred at 250 rpm for 30 min, and centrifuged. Polysaccharide-rich material sediment was washed with distilled water and dried at 50 °C for 24 h. Lignin-rich material precipitated from the liquid phase by adding distilled water (1:1 v/v), left for 24 h, centrifuged at 2755× g for 30 min, and dried at 50 °C for 24 h.	Enhanced Bioactive Liberation: ChCl:LA released more acidic protons than ChCl:Gly, actively cleaving the ester/ether bonds to completely dissolve the arabinan fraction (100% loss) and remove 77% of acid-soluble lignin. High-Purity Antioxidant Recovery: The recovered lignin-rich material exhibited a purity higher than 80% (78.50% Klason Lignin, 2.88% acid-soluble lignin), rich in guaiacyl and syringyl phenolic units suitable for bioactive materials. Cell Wall Deconstruction: Optimized ChCl:LA (130 °C, 90 min, 1:8 ratio) acted as a mild catalyst, dropping the cellulose lateral order index from 8.01 to 1.71 and destroying the amorphous matrix that traps bound phenolics.
Nkurunziza et al., 2025 [69]	BSG Freeze-dried, ground for 2 min in a 700 W blender, and completely deoiled via n-hexane extraction (1:10 w/v ratio, 50 °C, 20 h) under magnetic stirring.	ChCl:Gly (1:2) ChCl:U (1:2) (NADES) ChCl:MEA (1:1) (DES)	Direct biomass extraction with solvent. Downstream: centrifugation, dilution, and neutralization of the alkaline fraction to pH ~7 with 6 M HCl. Semi-purification via solid-phase extraction using C18-E cartridges activated with methanol/water; phenolic compounds eluted with 100% methanol, dried under nitrogen, and reconstituted in 50% methanol.	Premium radical scavenging: ChCl:MEA extracts exhibited outstanding antioxidant capacity via DPPH radical scavenging, matching the performance of the ascorbic acid positive control (50 µ/mL) and vastly outperforming ChCl:Gly, ChCl:U. Macrophage biocompatibility threshold: In vitro testing on RAW264.7 cells established a safe metabolic window for ChCl:MEA up to 100 µ/mL with no significant toxicity. Severe cytotoxicity (metabolic activity drop to 32%) was triggered at 316 µ/mL. Transcriptional inflammation silencing: At the maximum safe dose of 100 µ/mL, the glycosylated ferulate-rich ChCl:MEA extract significantly downregulated key pro-inflammatory gene expressions in LPS-stimulated macrophages, strongly suppressing Il-6 down to 46% and Il-1β to 42%.

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Pachulicz et al., 2025 [70]	BSG and MR Fresh BSG was stabilized at −20 °C and lyophilized (freeze-dried) for 72 h. BMR was processed in its industrially kiln-dried state. Both matrices were subjected to mechanical grinding using a 700 W blender for 2 min.	ChCl:Gly (1:2) (NADES)	Direct biomass extraction with solvent at 60 °C and 500 rpm for 3 h. Downstream: Double centrifugation at 14,000 rpm for 12 min, 3× dilution with Milli-Q water, and filtration through PTFE syringe filters. For scale-up bioassays, semi-purification via solid-phase extraction using an Amberlite XAD7HP polymeric resin column.	Premium Radical Scavenging: significantly higher antioxidant activity (DPPH and FRAP assays) and higher total phenolic content. Anti-Neurodegenerative Potency: hordatine fractions achieved complete inhibition of α-synuclein fibril elongation after 24 h at 37 °C.
Pariani et al., 2025 [71]	BSG Thermal stabilization in a ventilated oven at 60 °C for 24 h, followed by fine mechanical grinding using an electric grinder.	ChCl:LA (1:5) (NADES)	Two alternative methods: 1. Batch: 25 g BSG suspended in 250 mL solvent at 130 °C under magnetic stirring for 24 h. 2. MAE: 4 g BSG suspended in 40 mL solvent heated to 130 °C (5 min ramp, 6 h hold). Downstream: Gradual addition of ethanol over 2 h to precipitate the polysaccharide fraction, followed by centrifugation and filtration. Filtrate concentrated via rotary evaporation and stirred for 24 h at 4 °C to precipitate lignin.	Nutrient Pool Valorization: The isolated hydrothermal fraction was highly concentrated in monosaccharides, β-glucans, and soluble hemicelluloses and was successfully validated as a premium chemical-free growth medium for bio-fermentations, including PUFA-producing fungi and carotenoid/lipid-rich yeasts (<i>P. rhodozyma</i> and <i>Y. lipolytica</i>). Bioactive Lignin Functionalization: The microwave-assisted process achieved a 16.6% increase in functional phenolic hydroxyl groups within the isolated lignin, rising from 1.2 mmol/g to 1.4 mmol/g of vanillin equivalents.
Procentese et al., 2018 [72]	BSG Oven-dried at 40 °C, sieved to collect solids in the mechanical size range of 1–0.5 mm.	ChCl:Gly (1:2) (NADES) ChCl:EG (1:2) (DES)	3 h reaction time under variable tested configurations: temperatures at 60, 115, and 150 °C. Downstream: Centrifugation for 3 min at 5000 rpm until the aqueous phase ran clear, then dried at 38 °C. For enzymatic saccharification, the dried pretreated solid (10% w/v loading) was incubated in 0.1 M sodium citrate buffer at 50 °C and 180 rpm for 60 h, using Cellic CTec2 and Megazyme amylases.	Bioactive Phytochemical & Nutrient Pool: The initial processing of BSG isolates a substantial soluble fraction (25 wt.% of the total biomass) highly concentrated in β-glucans (immunomodulatory dietary fibers), soluble hemicelluloses, and phenylpropanoids.

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Provost et al., 2022 [73]	BSG Washed with water, oven-dried at 45 °C until constant weight, crushed and mechanically sieved to 0.2 mm. Subjected to an initial hydrothermal clearing treatment at 60 °C for 24 h with a solid-to-liquid ratio of 2.5% w/v.	ChCl:LA (1:10) ChCl:Gly (1:2) (NADES)	Reaction carried out under magnetic stirring in an oil bath for 24 h at two alternative temperature checkpoints: 60 °C or 80 °C. Downstream: Sintered-glass filtration. Solid washed with 95% ethanol, concentrated via vacuum rotary evaporation. The precipitated solid was separated by a second sintered-glass filtration and washed 3× with ultrapure water.	Phenolic Extraction Yield: High-resolution 2D-NMR mapped the baseline monomeric configuration of BSG lignin, showing a highly complex 35:36:29 G/H/S ratio. The explicit structural presence of the H-subunit (detected at 130 ppm) is directly correlated to low-molecular-weight dietary antioxidants and premium free-radical scavenging properties.
Roy et al., 2024 [74]	BSG Washed thoroughly, oven-dried at 60 °C for 24 h, mechanically blended, and sieved.	ChCl:LA (2:1) ChCl:TA (2:1) ChCl:MA (2:1) ChCl:Gly (2:1) ChCl:OA (2:1) (NADES) ChCl:EG (2:1) (DES) Note: DES used as modifiers in subcritical water (1:10 DES:water ratio) instead of pure solvents.	20 min reaction time at 180 °C and 40 bar. Downstream: Rapid cooling via internal chill line and depressurization, followed by double filtration through Whatman paper. Aliquots stored at 4 °C for liquid profiling or freeze-dried into stable solid powders for characterization and bioactivity bioassays.	Phenolic Extraction Yield: HPLC tracking mapped a monomeric profile dominated by 4-hydroxybenzoic acid and vanillic acid, with significant catechin content. Amino Acid Profile: Yields reached 108,112.87 mg/L (total) and 161.47 mg/L (free) using ChCl:TA. Glutamic acid and proline dominated overall, while leucine and valine led the essential amino acids. Bioactivity and Structure: Extracts showed high DPPH/ABTS antioxidant levels and strong antimicrobial activity against <i>E. coli</i> and <i>S. enterica</i> . FT-IR confirmed recovery of proteins (Amide A/B) and carbohydrates.

Table 1. Cont.

Author, Date	Matrix and Pre-Treatment	Extraction Solvent and Classification (DES or NADES)	Extraction Process	Principal Bioactive Outcomes
Santiago et al., 2025 [75]	BSG Ground in a knife mill and calibrated via an electromagnetic sieve machine.	ChCl:LA (1:2) ChCl:Gly (1:2) ChCl:CA (1:2) ChCl:OA (1:1) (NADES)	10 min screening under probe UAE (20 kHz, 8300 W/L) at 30 °C and an advanced ultrasonic bath process (55 W/L) was executed. Downstream: For screening, centrifugation (5000 rpm, 20 min) was used to separate the liquid extract for analysis. For the cleanup process, sequential extractions with hexane or ethanol were applied to the solid cake, which was then dried at 60 °C for 24 h to remove solvent residues.	Phenolic Extraction Yield: Yielding a total phenolic content (TPC) of 0.516 mg GAE/L within 5 min at 30 °C under the optimized sequential system (hexane pretreatment followed by ChCl:OA 1:1)
Wahlström et al., 2017 [76]	BSG Defatting of the raw matrix via Supercritical Carbon Dioxide extraction.	NaAcO:U (1:2; 1:3) KAcO:U (1:2; 1:3) NaForm:U (1:2; 1:3) (DES)	Heating set at 80 °C for acetate-based DES mixtures and 90 °C for formate-based DES mixtures. Implemented an optimized post-extraction mechanical step where the filtered solid residue was re-suspended and stirred vigorously in hot water before a final filtration stage. Downstream Purification: Laboratory-scale size-exclusion membrane dialysis using a 3.5 kDa molecular weight cut-off membrane to separate bulk proteins from low-molecular-weight DES components, followed by final product lyophilization (freeze-drying).	Protein Yields: Maximum protein extraction yield reached 79% at 10 wt% biomass consistency using 90 wt% NaAcO:U (1:2) after the optimized hot water washing step. The resulting dry solid concentrates contained 52.0 wt% to 54.7 wt% of total protein, alongside 19.8–21.4 wt% carbohydrates and 21.8–23.1 wt% total lignin. Amino Acid Profile: UPLC analysis showed selective dissolution of the prolamin fraction, resulting in high structural enrichment of glutamic acid and proline, while lysine and cysteine were highly reduced.

Abbreviations: two-dimensional nuclear magnetic resonance (2D-NMR); carbon-13 nuclear magnetic resonance (13C-NMR); acetic acid (AA); branched-chain fatty acids (BCFAs); betaine (Bet); brewers' malt rootlets (BMR); brewers' spent grain (BSG); brewers' spent hops (BSH); citric acid (CA); choline chloride (ChCl); ethylene glycol (EG); formic acid (FA); fructose (Fru); gallic acid equivalents (GAE); glucose (Glc); glycerol (Gly); guanidinium chloride (GuCl); high-intensity focused ultrasound (HIFU); high performance liquid chromatography (HPLC); half maximal inhibitory concentration (IC50); interleukin (IL); imidazole (Imi); lactic acid (LA); lipopolysaccharide (LPS); malic acid (MA); microwave-assisted extraction (MAE); malt dust (MD); maleic acid (MEA); malt rootlets (MR); natural deep eutectic solvents (NADES); sodium formate (NaForm); oxalic acid (OA); pyrolysis-gas chromatography-mass spectrometry (py-GC-MS); propylene glycol (PG); p-toluenesulfonic acid (PTSA); polyunsaturated fatty acid (PUFA); sorbitol (S); solid-phase extraction (SPE); sucrose (Suc); tartaric acid (TA); trehalose (Tre); ultra performance liquid chromatography (UPLC); urea (U); ultrasound-assisted extraction (UAE); valyl-isoleucyl-glutamyl-prolyl-arginine (VIEPR); weight percentage (wt%).

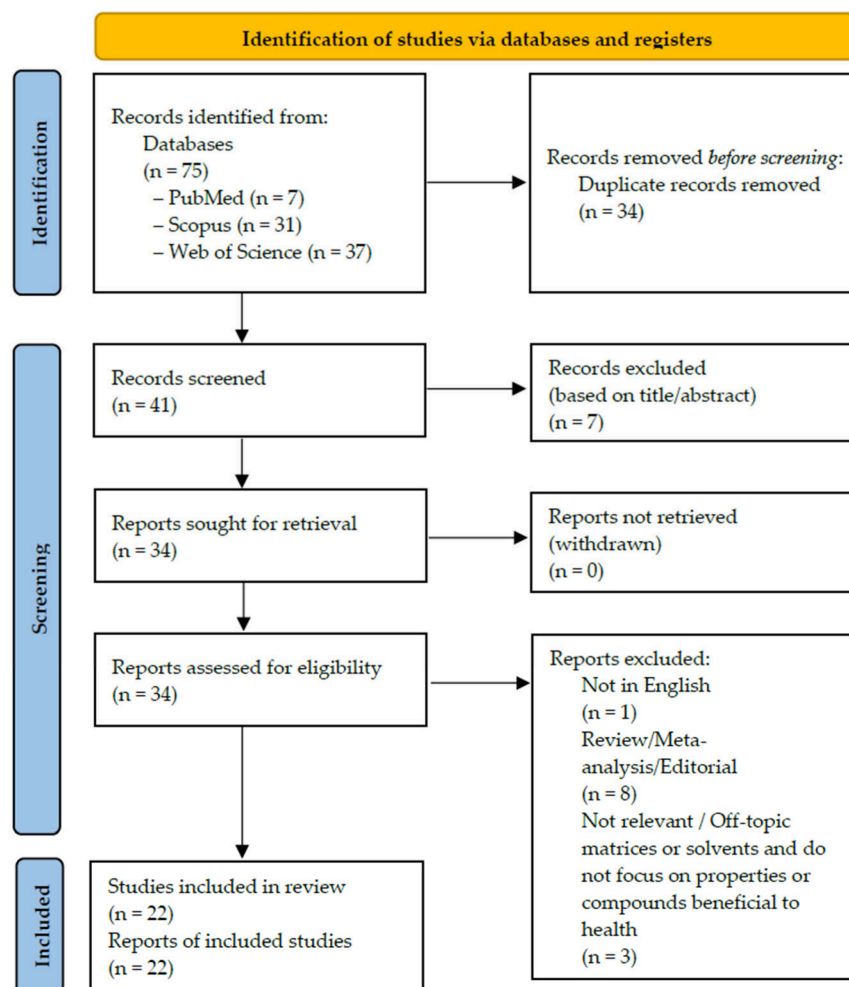


Figure 1. PRISMA 2020 flow diagram of the studies included.

3.3. Frequency Analysis of Solvent Systems and Extraction Techniques

A frequency analysis of the HBD paired with ChCl was performed (Figure 2). Acid-catalyzed systems (e.g., with H_2SO_4) were excluded from the heatmap. The most frequently employed donors were Gly and LA (16 and 12 occurrences, respectively), followed by EG (6 occurrences) and U (4 occurrences). Bet was the most frequently used alternative HBA after ChCl, with 8 occurrences and 6 different donors (U, Gly, EG, Glc, LA, Fru). Carboxylate salts (sodium acetate, potassium acetate, sodium formate) with U were used in one study.

Among the extraction techniques employed in the included studies, conventional heated maceration or hydrothermal extraction were the most frequently used, applied in 14 out of 22 studies. Among non-conventional technologies, MAE was predominantly applied with ChCl:LA and ChCl:Gly, while UAE and HIFU were used with a wider variety of formulations, including ChCl:OA, ChCl:Glc, and ChCl:Gly.

HBD	ChCl
Gly	16
LA	12
EG	6
U	4
OA	3
Glc	3
CA	3
MA	2
Imi	2
PG	2
Tre	2
FA	1
Suc	1
Fru	1
TA	1
S	1
AA	1
MEA	1

Figure 2. Heatmap showing the frequency of hydrogen bond donors (HBD) used with choline chloride (ChCl) in the 22 included studies. Abbreviations: acetic acid (AA); betaine (Bet); choline chloride (ChCl); citric acid (CA); ethylene glycol (EG); formic acid (FA); fructose (Fru); glucose (Glc); glycerol (Gly); hydrogen bond donor (HBD); imidazole (Imi); lactic acid (LA); malic acid (MA); maleic acid (MEA); oxalic acid (OA); propylene glycol (PG); sorbitol (S); sucrose (Suc); tartaric acid (TA); trehalose (Tre); urea (U).

4. Discussion

The systematic mapping of the 22 selected studies reveals that DES and NADES represent a significant change in the valorization of brewing by-products, transitioning from simple waste management to biorefinery processes. Rather than acting as passive, inert extractants, these green solvents function as active chemical agents capable of systematically dismantling the recalcitrant lignocellulosic architecture of BSG, BSH, and malt co-products. The discussion below interprets these findings by dissecting the molecular mechanisms of matrix deconstruction, evaluating the thermodynamic parameters of solvent-solute interactions, analyzing process engineering variables, and discussing the biological and nutraceutical value of the recovered phases.

4.1. Matrix Heterogeneity and Pre-Treatment Stabilization Dynamics

As summarized in Table 1, the investigated literature focuses predominantly on BSG, alongside specialized matrices such as BSH, MR, and MD. This biological heterogeneity directly stems from agricultural variations, crop seasonal dynamics, and primary industrial processing. According to foundational brewing by-products characterization models established in the literature [77,78], BSG typically comprises a complex, rigid assembly where

hemicellulose and lignin form the primary structural backbone. This specific polymer ratio dictates the mechanical resistance of the cell wall, meaning that baseline fluctuations in the raw material alter the accessibility of the matrix and require a precise tuning of the solvent's thermodynamic affinity to achieve reproducible extraction yields [79,80].

Because these sub-products leave the brewhouse with an high moisture content, often exceeding 70–80% [7], immediate stabilization is mandatory to halt microbial spoilage and enzymatic degradation of bioactive phytochemicals. The studies included in this review highlight diverse strategic approaches to matrix preparation, each profoundly altering the microscopic accessibility of the target molecules. For instance, methodologies like those applied by Allegretti et al. (2022) [55] utilize convection oven drying at moderate temperatures, typically around 40 °C to 60 °C, to lower water activity. While effective at halting microbial growth and enzymatic rancidity [81], controlled thermal stabilization is a double-edged sword. Extended exposure to even mild heat can trigger the partial oxidation or cleavage of sensitive, surface-associated free antioxidants [82], though it can simultaneously induce micro-fractures within the rigid carbohydrate domains, assisting subsequent solvent penetration by expanding the visible pore volume [83].

An alternative preservation strategy applied involves freeze-drying (lyophilization), as preferred in the experimental designs of Hadinoto et al. (2025) [61], Nkurunziza et al. (2025) [69], and Pachulicz et al. (2025) [70]. This cryogenic sublimation safeguards the intact structural integrity of fragile, highly thermolabile molecules and protects the overall aromatic and phenolic profile from oxidative degradation by operating under sub-zero vacuum conditions [84]. Nevertheless, raw brewing by-products literature [85] emphasizes that lyophilization introduces a substantial operational energy footprint—often requiring up to four to five times more energy than conventional convective drying—establishing a financial and logistical barrier that challenges its real-world scalability within a high-volume, low-margin industrial green biorefinery.

Beyond drying choices, alternative initial conditioning steps focus on structural or chemical purification, such as the autoclave thermal breakdown used by Allegretti et al. (2022) [55], the hydrothermal washing steps of Provost et al. (2022) [73], or the selective matrix defatting optimized via supercritical carbon dioxide by Wahlström et al. (2017) [76] and via organic n-hexane extraction by Nkurunziza et al. (2025) [69]. In all these pathways, managing the final water content remains a strict thermodynamic constraint. The addition of controlled water fractions is vital to reduce viscosity, yet thermodynamic modeling confirms that moisture must not cross a critical 30–40% threshold; exceeding this limit causes the essential hydrogen-bonding network between the HBA and donor to collapse, completely disrupting the supramolecular identity of the eutectic system [79,86].

Independent of the drying or washing method, mechanical milling to controlled mesh sizes emerges as a universal prerequisite across the extracted data. Grinding shatters the outer polymeric sheath of the brewing tissue, expanding the specific surface area available for solid–liquid contact [87]. This geometric reduction allows the viscous eutectic mixtures to deeply penetrate the microscopic pores, reducing internal mass transfer limitations and improving bioactive solvation kinetics [88].

4.2. Bioactive Outcomes: Solvent Chemistry and Extraction Technology Synergies

The extraction of bioactives from brewing by-products is governed by the thermodynamic affinity of the eutectic mixture. ChCl dominates as the primary HBA across the reviewed corpus due to its stability and biocompatibility. When paired with organic acids as HBD (e.g., LA or MA), these systems achieve high total phenolic content recoveries, particularly targeting ferulic and p-coumaric acids. Mechanistically, the localized acidity of these mixtures acts as a mild catalyst, cleaving the ester bonds that anchor hydroxycinnamic

acids to the arabinoxylan backbone of BSG without degrading the carbon skeleton [89]. Therefore, while DES and NADES offer clear benefits in terms of biocompatibility and environmental profile, their extraction performance is context-dependent and cannot be generalized as universally superior.

Conversely, pairing ChCl with polyols (Gly, EG) or sugars shifts the affinity toward free, non-esterified phenolics and fragile flavonoids (e.g., xanthohumol in BSH). However, these systems suffer from high intrinsic viscosity due to a dense, internal hydrogen-bonding network that restricts molecular diffusion [90]. To overcome this mass transfer limitation, the analyzed studies support water addition—typically ranging between 10% and 30% *v/v*, or specifically adapted depending on the target matrix, as reported in López-Linares et al. (2021) [63] and Pariani et al. (2025) [71]. This targeted dilution relaxes the solvent framework, lowering viscosity and accelerating solute-solvent complexation without disrupting the essential supramolecular structure of the eutectic.

To overcome the thermodynamic and viscosity barriers of eutectic mixtures, process intensification through non-conventional technologies is essential. Conventional solid-liquid extraction relies entirely on external thermal heating and mechanical stirring. While simple, this approach depends on slow molecular diffusion, often requiring prolonged processing times and elevated temperatures that risk the thermal oxidation of sensitive bioactives.

In contrast, UAE and MAE significantly accelerate extraction kinetics. UAE, utilized within the reviewed corpus by authors such as Santiago et al. (2025) [75], leverages acoustic cavitation. The propagation of ultrasound waves induces the violent collapse of micro-cavitation bubbles at the biomass-solvent interface, generating localized shockwaves and micro-jets. This physical phenomenon mechanically ruptures the recalcitrant lignocellulosic cell walls of BSG, forcing the viscous eutectic solvent directly into the microscopic pores and lowering mass transfer resistance [91].

On the other hand, MAE, as optimized within the reviewed corpus by López-Linares et al. (2021) [63], utilizes dielectric heating. Because DES (especially ionic mixtures based on ChCl) possess high ionic conductivity and polarizability, they absorb microwave radiation well. This leads to rapid, volumetric heating, causing the sudden vaporization of residual intracellular water. The resulting internal pressure shatters the plant cell wall from within, causing an instantaneous release of trapped polyphenols into the solvent phase [92]. This targeted heating reduces extraction times from hours to minutes, establishing MAE as a highly energy-efficient alternative for high-throughput biorefineries.

The multi-component nature of brewing by-products allows tuning the selectivity of DES toward either low-molecular-weight polyphenols or complex proteins. Acidic DES, particularly those incorporating LA or MA, selectively extract total phenolics [93] by cleaving the ester linkages that anchor hydroxycinnamic acids to the hemicellulose matrix, while leaving hydrophobic grain proteins (such as hordeins and glutelins) largely insoluble [94]. Conversely, shifting toward basic or amide-based HBD, such as U or specific amino acids, redirects the thermodynamic affinity toward the macromolecular nitrogenous fraction. These systems disrupt the hydrophobic interactions and disulfide bonds of the BSG, facilitating the co-extraction of proteins and bioactive peptides alongside free phenolics [61,76]. Beyond extraction yields, the dense hydrogen-bonding network of DES physically entraps and stabilizes sensitive molecules, such as ferulic acid from BSG or thermolabile xanthohumol from BSH [59].

4.3. Translational Potential in Sports Nutrition and Athletic Supplementation

It is important to emphasize that none of the included studies evaluated the effects of DES- or NADES-derived extracts on human subjects, athletic performance, or exercise recovery. The following discussion therefore interprets the characterized bioactive profiles

in light of established nutritional and physiological knowledge, clearly distinguishing between demonstrated *in vitro* bioactivities and potential translational applications that require future validation through *in vivo* and clinical studies.

Dietary supplementation may support overall metabolic health in the active population and could meet the physiological demands of competitive athletes by potentially accelerating glycogen resynthesis and mitigating exercise-induced muscle damage [95,96]. In this scenario, the transition toward natural, traceable, and bio-based ingredients is accelerating [97]. Using NADES presents a disruptive opportunity for the nutraceutical industry, because the resulting extracts are inherently non-toxic, biocompatible, and ready to use [98]. The eutectic phase can act directly as a stabilizing carrier, allowing the bioactive-rich liquid to be formulated straight into sports gels or functional recovery beverages. Moreover, the use of fully traceable brewing by-products aligns with the increasing demand for clean and controlled manufacturing processes in the supplement industry, reducing the risk of unintentional doping [99–102].

Physical exercise increases reactive oxygen species production, leading to transient oxidative stress and skeletal muscle micro-damage [41–43]. The ISSN position stand acknowledges that foods and beverages rich in polyphenols are preferred antioxidant sources, while dietary supplementation is best reserved for periods of high training distress or inadequate dietary intake [103]. The reviewed corpus demonstrates that polyphenols extracted via eutectic mixtures provide a rich antioxidant pool. Chromatographic analyses highlight a recovery of key hydroxycinnamic acids—specifically ferulic and p-coumaric acids—as optimized in the work of Masiello et al. (2026) [64] and Bleus et al. (2024) [57]. These phenolic monomers, alongside depolymerized lignin-derived fractions that exhibit highly efficient radical-scavenging activity in the studies by Antoun et al. (2024) [56] and Cassoni et al. (2023) [58], demonstrate significant potential to neutralize free radicals *in vitro*, which may mitigate oxidative stress mechanisms and post-exercise systemic inflammation [104–106].

Furthermore, the recovery of xanthohumol from BSH using Gly and organic acid-based mixtures, as detailed by Grudniewska et al. (2020, 2023) [59,60], adds a bioactive component. While xanthohumol is a well-documented modulator of the Nrf2 pathway in a cellular model [107,108], whether such phenolic profiles can reduce post-exercise muscle soreness requires confirmation in clinical settings. Additionally, *in vitro* assays validate the safety of these extracts, with Nkurunziza et al. (2025) [69] showing anti-inflammatory potential through the significant downregulation of pro-inflammatory cytokines Il-6 and Il-1 β .

Alternative bioconversion pathways further complement this lipophilic cellular protection. In the system developed in the study of Allegretti et al. (2022) [55], eutectic-treated biomass was utilized to yield high concentrations of carotenoids, specifically astaxanthin—a fat-soluble antioxidant—alongside polyunsaturated fatty acids (PUFAs) like linoleic acid and branched-chain fatty acids (BCFAs). Incorporating these essential lipids may support cell membrane integrity against exercise-induced lipid peroxidation, mitigate systemic inflammatory cascades, and aid in lipid-mediated metabolic recovery [109,110]. The ISSN position stand recommends at least 1000 mg/day of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for antioxidative effects, with doses of 2000–6000 mg/day to attenuate muscle damage [103]. The lipid yields obtained from BSG (up to 547 mg/L), with linoleic acid representing 72% of the total, are well below these recommended levels. While benchmarking these yields against ISSN guidelines illustrates the raw potential of BSG, *in vitro* extraction values cannot be directly translated into dietary recommendations without accounting for raw material conversion rates and *in vivo* bioavailability.

Beyond pure antioxidant protection, the co-extraction of proteinaceous fractions from brewing by-products offers plastic and regenerative support for muscle tissue. Using amino

acid- or amide-based solvents, such as the U-containing over 50 wt% of total protein. The protein concentrate obtained by Hadinoto et al. [61] contained approximately 42% essential amino acids, with leucine among the most abundant (7.19%). This specific profile, rich in branched-chain amino acids (BCAA), is qualitatively relevant for stimulating muscle protein synthesis via the mTOR pathway [111–113]. The ISSN position stand indicates that essential amino acids stimulate muscle protein synthesis at dosages ranging from 1.5 to 18 g, with a higher leucine percentage required to maximally stimulate synthesis [114]. While these essential amino acid profiles highlight the raw nutritional quality of the recovered fractions, they reflect hydrolyzed protein composition rather than free-form bioavailability, and bridging the gap between bench-scale yields and human athletic requirements strictly requires future validation through digestibility, formulation, and clinical absorption studies. Additionally, the functional value of this macromolecular pool is further enhanced by the identification of bioactive peptides, such as the specific sequence valyl-isoleucyl-glutamyl-prolyl-arginine (VIEPR) discovered through the sequencing performed by Hernández-Corroto et al. [62].

Simultaneously, this progressive deconstruction of the biomass cell wall liberates functional complex carbohydrates alongside the nitrogenous fraction. As observed in the study by López-Linares et al. (2021) [63], optimized eutectic extraction leaves a carbohydrate-dense residue ideal for upcycling prebiotic arabinoxylans. These non-digestible fractions are further characterized in the studies by Pariani et al. (2025) [71] and Procentese et al. (2018) [72], which successfully isolated soluble liquid phases highly concentrated in hemicelluloses and β -glucans. From a nutritional standpoint, these polysaccharides may act as immunomodulators and prebiotics [115,116]. The EFSA considers a dietary fibre intake of 25 g/day adequate for normal laxation in adults [117]. While no specific recommendations exist for prebiotic β -glucans or arabinoxylans in athletic populations, the fibre-rich fractions obtained from BSG may contribute to overall fibre intake. By potentially stimulating beneficial gut microbiota [118,119], these prebiotic fractions represent candidates for further research regarding the gut-muscle axis and intestinal mucosa support during intensive training.

Ultimately, the capacity of NADES to simultaneously extract, stabilize, and deliver these diverse molecular families directly from brewing by-products—merging polyphenols, proteins, essential lipids, and prebiotic fibers into a single biocompatible liquid phase—could offer a route for formulating multi-component, and ready-to-use functional supplements, but requires further validation through in vivo studies to confirm bioavailability, safety, and efficacy in athletic populations.

4.4. Safety Considerations

The intrinsic composition of the brewing by-products themselves poses safety concerns. BSG derives from barley, which contains gluten proteins called hordeins that cause adverse reactions in individuals with celiac disease and related conditions [120]. The presence of gluten in the final extracts has implications for celiac patients, as studies have shown that hordeins are enriched in BSG compared to the starting malt, making BSG unsafe for consumers with celiac disease [121]. Furthermore, BSG has high moisture content and is prone to rapid microbial spoilage, which can lead to mycotoxin contamination, including ochratoxin A and deoxynivalenol [122,123].

Beyond the risks associated with the raw material, the safety of the extraction solvents themselves must also be critically evaluated. Even NADES formulations—although composed of natural metabolites—cannot be automatically considered fully food-grade or safe for ingestion. The non-volatile nature of these solvents means that they remain in the final extract, raising concerns about the potential co-ingestion of antinutritional factors.

For example, the use of OA is particularly concerning because it is a well-established antinutrient and a risk factor for kidney stone formation [124]. Additionally, although individual NADES components (such as ChCl, LA, or organic acids) are generally recognized as safe at current intake levels when used as food additives or flavourings [125,126], the safe status and acceptable daily intake (ADI) values established for isolated components cannot be directly extrapolated to the intact eutectic phase. However, the co-ingestion of multiple components in a concentrated eutectic mixture—potentially at doses exceeding those from normal dietary exposure—raises unresolved questions regarding cumulative toxicity, metabolic interactions, and gastrointestinal tolerance.

This issue is further compounded when considering that the highest extraction yields for specific bioactive compounds were often achieved using synthetic, non-food-grade DES systems. For instance, the maximum protein recovery was obtained with a NaAcO:U [76] and the highest catechol concentration resulted from ChCl:EG acidified with 0.9% H₂SO₄ [66]. These findings underscore the need to distinguish between systems optimized for extraction efficiency and those suitable for food or nutraceutical applications.

Finally, acidic DES may also co-extract heavy metals (e.g., lead, cadmium, arsenic) present in the raw biomass [127,128].

4.5. Limitations and Future Perspectives

A limitation of this scoping review is the absence of a formal risk-of-bias assessment, consistent with the PRISMA-ScR framework. The methodological quality of the included studies was not systematically evaluated, and this should be considered when interpreting the findings.

Despite the translational potential of DES and NADES-mediated extraction, several technical and regulatory challenges must be addressed before wide-scale commercialization in sports nutrition. Scientifically, the high viscosity of eutectic mixtures remains a primary limitation, as it restricts mass transfer and slows the diffusion of target compounds from the solid matrix to the bulk solvent. While water addition and temperature elevation can reduce viscosity, these strategies require careful optimization; excessive dilution disrupts the hydrogen-bonding network that defines the eutectic system [129], while heating may degrade thermolabile bioactives [130]. To overcome these constraints, advanced energy-assisted technologies such as MAE and UAE have been employed to enhance extraction efficiency and reduce processing times. However, most studies remain at laboratory scale, and the industrial scalability of these combined approaches is still unproven, as energy consumption and equipment costs may offset the environmental benefits of the green solvents themselves.

The overall environmental sustainability of DES and NADES-based processes depends not only on extraction efficiency but also on solvent preparation, recyclability, and the energy required for process intensification. DES and NADES systems, even with high recycling rates, performed worse than water or ethanol in most environmental impact categories, primarily due to the energy and raw materials required for solvent preparation; therefore, while DES and NADES may be an option to avoid more harmful solvents, they should not substitute abundant and non-dangerous ones like water or ethanol-water mixtures [131].

Furthermore, while many formulations are described as “ready-to-use,” certain downstream applications still require water-induced precipitation or solid-phase extraction to isolate specific bioactives (e.g., purified xanthohumol or isolated peptides), which can partially offset the green character of the process.

Regarding regulatory and clinical safety, although individual NADES components (like CHCl, Gly, or organic acids) are generally recognized as safe, comprehensive *in vivo*

toxicological evaluations of chronic, high-dose ingestion of the intact eutectic phases are still scarce. In particular, there is a critical need to verify their safety profile regarding intestinal toxicity *in vivo*, as the surfactant-like behavior or high osmolarity of certain eutectic mixtures could potentially disrupt the gut mucosal barrier or alter the microbiota of athletes. Additionally, the organoleptic properties of these mixtures present a challenge for consumer compliance, as the inherent taste of certain NADES components may not be pleasant. This potential lack of palatability must be addressed or masked before these extracts can be realistically incorporated into sports supplements.

Future research should prioritize the following areas: (i) scaling up DES and NADES extraction from batch to continuous industrial processes, with a focus on energy-efficient solvent recovery and recycling systems; (ii) conducting comprehensive *in vivo* toxicological studies and clinical trials to validate the safety, bioavailability, and efficacy of NADES-derived extracts in athletic populations; (iii) establishing regulatory frameworks and maximum residue limits for NADES components in food-grade applications; (iv) implementing mandatory screening for mycotoxins and heavy metals in raw brewing by-products and final extracts; (v) standardizing organoleptic profiles and shelf-life stability of liquid extracts for consumer acceptability; and (vi) performing life-cycle assessments to evaluate the true environmental footprint of DES and NADES synthesis and recovery.

5. Conclusions

This scoping review provides a mapping of the current evidence on DES- and NADES-mediated extraction of bioactives from brewing by-products, with a focus on extraction yields, bioactive profiles, and their potential relevance for sports nutrition.

Eutectic solvents demonstrate efficiency in disrupting the recalcitrant lignocellulosic matrix of brewing by-products. These systems yield phenolic profiles dominated by ferulic and *p*-coumaric acids, especially when coupled with MAE or UAE. Concurrently, U- or polyol-based mixtures isolate protein fractions and functional peptides, offering an alternative to petroleum-derived organic solvents.

The resulting extracts exhibit promising *in vitro* bioactivities, including radical-scavenging and anti-inflammatory properties, as well as essential amino acid profiles that may be relevant for muscle protein synthesis. Additionally, the co-extraction of prebiotic fibers (β -glucans and arabinoxylans) suggests potential immunomodulatory benefits, though these effects require *in vivo* validation.

However, several safety and regulatory gaps remain. The presence of gluten in BSG-derived extracts, the potential co-ingestion of antinutritional factors (e.g., oxalic acid), and the lack of toxicological data on intact eutectic phases underscore the need for further research before these extracts can be considered for human consumption. Addressing these gaps will be essential to determine whether DES- and NADES-derived extracts can ultimately transition from promising *in vitro* candidates to safe and effective ingredients for functional foods and sports nutrition.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/appliedchem6040068/s1>, Supplementary File S1: PRISMA-ScR-Fillable-Checklist and List of Included Studies and Excluded Studies with Reason.

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Abbreviations

The following abbreviations are used in this manuscript:

2D-NMR	Two-dimensional nuclear magnetic resonance
¹³ C-NMR	Carbon-13 nuclear magnetic resonance
AA	Acetic acid
BCFAs	Branched-chain fatty acids
Bet	Betaine
BMR	Brewers' malt rootlets
BSG	Brewers' spent grain
BSH	Brewers' spent hops
BSY	Brewers' spent yeast
CA	Citric acid
ChCl	Choline chloride
DES	Deep eutectic solvents
DHA	Docosahexaenoic acid
EG	Ethylene glycol
EPA	Eicosapentaenoic acid
FA	Formic acid
Fru	Fructose
GAE	Gallic acid equivalents
Glc	Glucose
Gly	Glycerol
GuCl	Guanidinium chloride
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HIFU	High-intensity focused ultrasound
HPLC	High performance liquid chromatography
IC ₅₀	Half maximal inhibitory concentration
IL	Interleukin
Imi	Imidazole
ISSN	International Society of Sports Nutrition
LA	Lactic acid
LPS	Lipopolysaccharide
MA	Malic acid
MAE	Microwave-assisted extraction
MD	Malt dust
MEA	Maleic acid
MR	Malt rootlets
NADES	Natural deep eutectic solvents
NaForm	Sodium formate
OA	Oxalic acid
OSF	Open Science Framework
PG	Propylene glycol
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PRISMA-ScR	PRISMA extension for Scoping Reviews
PTSA	p-Toluenesulfonic acid
PUFA	Polyunsaturated fatty acid
py-GC-MS	Pyrolysis-gas chromatography-mass spectrometry
ROS	Reactive oxygen species
S	Sorbitol
SPE	Solid-phase extraction
Suc	Sucrose
TA	Tartaric acid
Tre	Trehalose
U	Urea
UAE	Ultrasound-assisted extraction
UPLC	Ultra-performance liquid chromatography
VIEPR	Valyl-isoleucyl-glutamyl-prolyl-arginine
wt%	Weight percentage

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