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Eco-Efficient Ultrasound-Assisted Extraction of Polyphenols from Date Palm Kernels Using Natural Deep Eutectic Solvents: Antioxidant and Antibacterial Activities

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Featured Application

Hydrated natural deep eutectic solvents combined with sonotrode ultrasound-assisted extraction enable the sustainable production of ready-to-use polyphenol-rich liquid extracts from date kernels. The obtained extracts exhibit enhanced antioxidant and antimicrobial properties, supporting their application as multifunctional natural additives for food preservation, formulation, and agro-industrial by-product valorization.

Abstract

This study optimized the extraction of polyphenols from *Phoenix dactylifera* L. kernels by combining hydrated natural deep eutectic solvents (NADESs) with sonotrode ultrasound-assisted extraction (S-UAE), aiming to obtain a ready-to-use antioxidant and antimicrobial liquid extract. A sequential optimization strategy based on Box–Behnken designs identified the solvent-to-solid ratio as the main extraction driver. The optimal conditions were 55% hydrated NADES, a 5 mL/g solvent-to-solid ratio, 60% amplitude, 170 J/mL specific energy, and an 80% duty cycle. Under these conditions, experimental concentrations reached 309.78 mg/L total polyphenols and 265.81 mg/L flavan-3-ols, the predominant phenolic family. Compared with conventional agitation extraction, optimized S-UAE increased total polyphenols by 65.78%, flavan-3-ols by 68.65%, hydroxycinnamic acids by 48.47%, and flavonols by 35.05%. Antioxidant activity was markedly enhanced, reaching 15.47 and 14.70 mg Trolox eq./L in DPPH and FRAP assays, respectively, representing approximately 16-fold and 9-fold improvements over agitation extraction. The extract also showed antibacterial activity, producing inhibition zones of 16.0 mm against *Bacillus* sp. and 12.0 mm against *Pseudomonas fluorescens*, while complete inhibition was achieved against several strains in broth assays. These findings demonstrate that hydrated NADESs coupled with S-UAE is an efficient and scalable green strategy for valorizing date kernels into multifunctional polyphenol-rich food additives.

Keywords: date seeds; natural deep eutectic solvents; sonotrode ultrasound-assisted extraction; polyphenol valorization

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1. Introduction

The date palm (*Phoenix dactylifera* L.) is one of the most extensively cultivated crops in arid and semi-arid regions, playing a vital role in the economy and dietary habits of these areas. However, the industrial processing and commercialization of dates generate a significant amount of agricultural waste [1,2]. Date palm kernels, which represent approximately 10% of the total fruit weight, are the primary co-product of this industry. Often discarded or used as low-value animal feed, these kernels are actually a rich source of bioactive compounds, particularly polyphenols, which possess potent antioxidant and antimicrobial properties [3,4]. Polyphenols from date kernels have attracted considerable attention due to their remarkable antioxidant and antimicrobial properties. Their antioxidant activity contributes to preventing lipid oxidation and preserving food quality, whereas their antimicrobial potential may inhibit the growth of foodborne pathogens and spoilage microorganisms. These bioactivities make date kernel extracts promising natural ingredients for food preservation and functional food applications.

In line with sustainable development, the extraction of these high-value compounds must align with the principles of Green Chemistry [5,6]. Traditional extraction methods often rely on large volumes of toxic organic solvents, which pose environmental and health risks. To address this, natural deep eutectic solvents (NADESs) have emerged as a revolutionary class of eco-friendly solvents [7,8]. Composed of natural primary metabolites (such as sugars, amino acids, or organic acids), NADESs offer advantages like low toxicity, biodegradability, and a remarkable ability to solubilize complex plant matrices, often surpassing the efficiency of conventional solvents [9,10].

To further enhance extraction efficiency and reduce processing time, Ultrasound-Assisted Extraction (UAE) has been widely adopted. UAE utilizes acoustic cavitation to disrupt plant cell walls, facilitating the mass transfer of target molecules into the solvent [11,12]. The synergy between UAE and NADESs represents a powerful, green approach to valorizing agri-food co-products [9,13–15]. This synergy has been reported to significantly outperform conventional extraction techniques across various agri-food matrices [16]. Ultrasound-driven cavitation generates intense localized energy that fractures the rigid architecture of some of these matrices, providing a larger surface for solvent interaction [17]. By mitigating the flow resistance typically associated with viscous NADESs, ultrasound promotes a deeper infiltration into the biomass. This allows the NADES to establish robust hydrogen-bond networks with target polyphenols, maximizing their solubility. Such synergy not only optimizes the overall extraction efficiency but also ensures a higher preservation of the extract's biological activity than conventional single-stage techniques [18,19]. Airouyuwa et al. [7,13] employed UAE with different NADESs (carboxylic acid-based and sugar-based NADESs) for the extraction of bioactive compounds from date seeds, obtaining in both cases higher yield in total phenolic and flavonoid content compared to those obtained using a conventional extraction process (80% ethanol and methanol). Chen et al. [19] showed that the use of a choline chloride:d-fructose NADESs combined with UAE technology was an efficient method to extract polyphenols from flaxseed meal, achieving the highest recovery rates for both free phenolics and bound phenolics and superior antioxidant activity and hypoglycemic activity compared with conventional extraction methods (70% ethanol). Xu et al. [16] highlighted the strong potential of UAE with NADESs for the valorization of agro-food waste based on the notable advantages of these methods such as higher extraction yields, reduced energy and solvent consumption, shorter processing times and enhanced selectivity compared to conventional extraction methods. However, it is evident that a 'one-size-fits-all' approach is not feasible for selecting NADES compositions or extraction parameters. The optimization process is inherently dictated by the structural complexity of the biomass and the physicochem-

ical properties of the molecules of interest. Although common benchmarks, such as an ultrasonic power between 300 and 400 W, a 40 °C thermal setting, and a 30 min duration, often yield favorable results [16,19], these figures must be treated as initial reference points. Rigorous, substrate-specific validation remains essential not only to achieve peak extraction performance but also to preserve their biological activities.

Therefore, despite the known benefits of these technologies, optimizing the extraction parameters is crucial to maximizing yield and bioactivity. Accordingly, this work aimed to optimize the extraction of polyphenols from date kernels by coupling natural deep eutectic solvents (NADESs) with sonotrode-assisted ultrasound-assisted extraction (S-UAE), focusing not only on maximizing polyphenol recovery but also on directly obtaining a polyphenol-rich liquid extract within the NADES matrix, where the eutectic mixture acts as a functional carrier. In addition, the extraction process was optimized to improve kinetic efficiency and extract manageability, while assessing the potential of the resulting fluid as a ready-to-use liquid food additive with dual antioxidant and antimicrobial functionality.

2. Materials and Methods

2.1. Plant Material

Date kernels (*Phoenix dactylifera* L. cv. Deglet Noir) obtained from date fruits in the Tamar ripeness stage, were supplied by the company Bernabe Biosca Alimentación S.A. (Campello, Alicante, Spain). The kernels were dehydrated (for 48 h at 55 °C), ground in a stone mill, and sieved to a particle size of less than 0.210 mm. The ground kernel was vacuum-packaged and stored in a dark place.

2.2. Polyphenol Extraction Procedures

2.2.1. Preparation of Natural Deep Eutectic Solvent (NADES)

The natural deep eutectic solvent (NADES) was prepared by mixing fructose (hydrogen bond acceptor) with malic acid and glycerol (hydrogen bond donors) at a 1:1:1 molar ratio. The mixture was stirred and heated at 60 °C until a clear, homogeneous liquid was obtained. During synthesis, 30% (*w*/*w*) of distilled water was added to facilitate the mixing and dissolution of the components. The NADES was stored under refrigeration (4 °C) until use.

2.2.2. Sonotrode-Ultrasonic Assisted Extraction (S-UAE)

For extraction, the NADES was diluted with water (15–55%, *w*/*w*). Therefore, the extraction was performed utilizing either hydrated NADES (55%, *w*/*w*) or NADES aqueous solutions (15% and 35%, *w*/*w*). The samples were weighed into a 50 mL Falcon tube, and 25 mL of the corresponding NADES solution was added; the sample weight was adjusted according to the specific liquid-to-solid ratio (mL/g) used (3:1, 5:1, 10:1, 15:1, 40:1, or 70:1). The tube was vortexed and submitted to ultrasound-assisted extraction. An ice bath was continuously used during operation to prevent thermal degradation, ensuring the extraction temperature did not exceed 40 °C. For S-UAE, a UP400St ultrasonic processor (400 W; 24 kHz) (Hielscher Ultrasonics GmbH, Teltow, Germany) coupled to an S24d7 sonotrode was used. The ultrasonication conditions can be observed in Table 1. After the sonication process, the samples were centrifuged for 5 min at 7200× *g* at 4 °C. Subsequently, the supernatant was vacuum filtered through filter paper and stored in amber vials under refrigeration until the purification process and antimicrobial assays.

Table 1. Four-factor Box–Behnken experimental design matrix (expressed in dimensional and dimensionless independent variables) and experimental responses obtained for Phase I optimization.

		Independent Variables				Dependent Variables			
		A (%)	SE (J/mL)	NADES (%)	R (mL/g)	TPC (mg/L)	F3O (mg/L)	HCA (mg/L)	FLV (mg/L)
No	Block	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3	Y_4
1	1	25 (−1)	20 (−1)	35 (0)	40 (0)	32.31	27.09	2.21	2.53
2	1	75 (+1)	20 (−1)	35 (0)	40 (0)	27.71	23.04	2.02	2.40
3	1	25 (−1)	140 (+1)	35 (0)	40 (0)	33.94	29.01	2.21	2.35
4	1	75 (+1)	140 (+1)	35 (0)	40 (0)	44.14	38.34	2.59	2.83
5	1	50 (0)	80 (0)	15 (−1)	10 (−1)	106.06	91.77	6.51	6.59
6	1	50 (0)	80 (0)	55 (+1)	10 (−1)	111.66	91.75	8.39	10.05
7	1	50 (0)	80 (0)	15 (−1)	70 (+1)	20.67	17.90	1.24	1.35
8	1	50 (0)	80 (0)	55 (+1)	70 (+1)	16.66	13.61	1.25	1.73
9	1	50 (0)	80 (0)	35 (0)	40 (0)	37.83	32.29	2.48	2.69
10	2	25 (−1)	80 (0)	35 (0)	10 (−1)	129.84	109.36	9.09	10.14
11	2	75 (+1)	80 (0)	35 (0)	10 (−1)	137.18	117.59	8.63	9.64
12	2	25 (−1)	80 (0)	35 (0)	70 (+1)	17.95	15.80	0.92	1.11
13	2	75 (+1)	80 (0)	35 (0)	70 (+1)	23.04	19.95	1.35	1.55
14	2	50 (0)	20 (−1)	15 (−1)	40 (0)	40.79	36.04	2.22	2.28
15	2	50 (0)	140 (+1)	15 (−1)	40 (0)	37.96	33.65	1.98	2.04
16	2	50 (0)	20 (−1)	55 (+1)	40 (0)	38.24	33.51	1.86	2.66
17	2	50 (0)	140 (+1)	55 (+1)	40 (0)	45.97	40.57	2.25	2.83
18	2	50 (0)	80 (0)	35 (0)	40 (0)	39.78	33.94	2.64	2.83
19	3	25 (−1)	80 (0)	15 (−1)	40 (0)	35.20	31.11	1.92	1.89
20	3	75 (+1)	80 (0)	15 (−1)	40 (0)	35.41	31.36	1.81	1.98
21	3	25 (−1)	80 (0)	55 (+1)	40 (0)	21.62	17.87	1.49	2.20
22	3	75 (+1)	80 (0)	55 (+1)	40 (0)	30.84	26.04	2.08	2.51
23	3	50 (0)	20 (−1)	35 (0)	10 (−1)	79.96	65.97	6.56	6.83
24	3	50 (0)	140 (+1)	35 (0)	10 (−1)	88.54	73.73	6.62	7.53
25	3	50 (0)	20 (−1)	35 (0)	70 (+1)	19.42	16.75	1.26	1.32
26	3	50 (0)	140 (+1)	35 (0)	70 (+1)	18.65	15.83	1.24	1.51
27	3	50 (0)	80 (0)	35 (0)	40 (0)	29.56	24.83	2.09	2.55
28	1	50 (0)	80 (0)	35 (0)	40 (0)	30.93	26.82	1.79	2.10
29	2	50 (0)	80 (0)	35 (0)	40 (0)	30.69	26.17	1.94	2.32
30	3	50 (0)	80 (0)	35 (0)	40 (0)	26.93	22.68	1.83	2.30

X_1: Amplitude (%); X_2: Specific energy (J/mL); X_3: NADES concentration (%; *w*/*w*); and X_4: Solvent-to-solid ratio (mL/g). Coded factors are dimensionless and restricted to the [−1, 0, +1] operational space. Experimental responses (Y) are reported as individual measurements for each specific design matrix coordinate. TPC: Total (poly)phenolic content; F3O: Flavan-3-ols; HCA: Hydroxycinnamic acids; FLV: Flavonols. Fixed ultrasonic variables: duty cycle: 100%.

2.3. Polyphenols Identification and Quantification

Following each extraction process, the obtained extract was purified prior to HPLC injection. For this purpose, a C18 solid-phase extraction (SPE) cartridge was employed, as previously described by Lucas-González et al. [12]. After activating the cartridge with methanol, distilled water, and HCl (0.01 N), an aliquot of the sample was loaded. The cartridge was washed with water to remove polar interferences, and the target polyphenols were subsequently eluted with acidified methanol (methanol:formic acid, 99:1, *v*/*v*). A 20 µL aliquot of the purified extract was injected into an HPLC system (Hewlett-Packard HPLC 1200 series) equipped with a C18 reversed-phase column Mediterranean Sea 18 (Teknokroma, Barcelona, Spain) (25 × 0.4 cm; 5 µm). The mobile phases consisted of acetonitrile (A) and acidified water (B; water:formic acid, 99:1, *v*/*v*). Three wavelengths were used to monitor the polyphenolic signals: 280, 325 and 360 nm. For peak identification, retention times and UV-Vis absorbance spectra were compared with those of 50 commercial

standards analyzed under identical chromatographic conditions. All commercial standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Extrasynthese (Genay, France).

Calibration curves for the targeted compounds exhibited high linearity ($R^2 > 0.996$). When a specific standard was unavailable, the absorbance spectrum was utilized to classify the compound into its corresponding polyphenolic subfamily. Unidentified hydroxycinnamic acids were quantified using caffeic acid as a reference standard, unidentified flavan-3-ols were quantified against catechin, and unidentified glycosylated flavonols were quantified using quercetin-3-glucoside.

2.4. Sequential Experimental Design

The extraction of polyphenols from date seeds was optimized using a two-step sequential experimental design. The optimization process aimed to maximize the concentration of polyphenols per mL of extraction medium.

2.4.1. Phase I Screening Design (Four-Factor Box–Behnken)

Based on previous laboratory work () and the literature, an initial Box–Behnken design (BBD) was performed to evaluate the four most influential factors: amplitude (X_1; 25–75%), specific energy (X_2; 20–100 J/mL), hydrated NADES concentration (X_3; 15–55% *w*/*w*), and solvent-to-solid ratio (X_4; 10–70 mL/g). Each factor was studied at three levels (−1, 0, +1). The design consisted of 27 runs plus three additional center points, yielding 30 total runs. To account for potential experimental variability, the design was blocked into three distinct blocks carried out on three different days, with a fresh batch of hydrated NADESs synthesized for each block. The monitored responses were total polyphenolic compounds (Y_1), total flavan-3-ols (Y_2), total hydroxycinnamic acids (Y_3), and total flavonols (Y_4), all expressed as mg/L and all calculated as the sum of all individual phenolic compounds and the sub-families of compounds quantified via High-Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD).

Unifactorial Optimization of Solvent-to-Solid Ratio

After the Phase I screening design (four-factor Box–Behnken), the solvent-to-solid ratio was further optimized using a one-factor-at-a-time approach. The solvent-to-solid ratio was varied over a range of 3–15 mL/g while keeping all other parameters constant: hydrated NADES concentration (55%, *w*/*w*), 75% amplitude, 100% duty cycle, and 140 J/mL, which corresponded to the optimal values obtained from the first design. The relative mass recovery of the target polyphenols was used as the selection parameter to determine the best solvent-to-solid ratio to be fixed as the optimal value and subsequently applied in the Phase II design.

2.4.2. Phase II Screening Design (Three-Factor Box–Behnken)

To complete the full optimization process, a new three-factor Box–Behnken design was carried out to optimize exclusively the S-UAE parameters. The three factors were: amplitude (X_1: 60–80%), specific energy (X_2: 60–170 J/mL), and duty cycle (X_3: 20–100%). Fixed variables were the solvent-to-solid ratio (5:1 mL/g) and a 55% hydrated NADES. The design included 15 runs (3 center points). The monitored responses were identical to those in the Phase I screening design: total polyphenol content (Y_1), total flavan-3-ols (Y_2), total hydroxycinnamic acids (Y_3) and total flavonols (Y_4), all expressed as mg/L and all calculated as the sum of all individual phenolic compounds and the sub-families of compounds quantified via HPLC-DAD.

2.5. Evaluation Efficiency of S-UAE

The extraction efficiency of the S-UAE method was evaluated by comparing it with the conventional agitation-assisted extraction method. For this purpose, two extracts were prepared under their respective optimized conditions: (i) the extract obtained using the optimized S-UAE conditions established after the Phase II experimental design, hereafter referred to as DKE-U (date kernel extract obtained by ultrasound-assisted extraction), and (ii) the extract obtained using the conventional agitation-assisted extraction method, referred to as DKE-A (date kernel extract obtained by agitation-assisted extraction). The efficiency of S-UAE was calculated based on the increase in polyphenol recovery, using DKE-A as the baseline reference for comparison with DKE-U.

2.6. Antioxidant Activity of DKE-U and DKE-A

The antioxidant activity of the purified extract obtained in Section 2.4 was assessed using two different antioxidant assays. Therefore, the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH assay) was performed as described by Brand-Williams et al. [20]. For that, a volume of 50 µL of a purified extract was placed in a cuvette, and 2 mL of $6{\cdot}10^{-5}$ mol/L methanolic solution of DPPH was added. The mixtures were well shaken in a Vortex (2500 rpm) for 1 min and then placed in a dark room for 15 min. The decrease in absorbance at 517 nm was determined. The results were expressed as mg Trolox Equivalents/L of extract. The Ferric Reducing Antioxidant Power (FRAP assay) was measured by means of the potassium ferricyanide-ferric chloride method according to the methodology described by Oyazu [21]. One mL of a purified extract was added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, after which 2.5 mL trichloroacetic acid (10%) was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL water and 0.5 mL 0.1% $FeCl_3$. The absorbance at 700 nm was measured after allowing the solution to stand for 30 min. The results were expressed as mg Trolox Equivalents/L of extract.

2.7. Antibacterial Activity

2.7.1. Microorganisms and Culture Conditions

The antibacterial activity of date kernel extracts was evaluated against five bacterial strains, including three Gram-positive bacteria (*Listeria innocua* CECT 910, *Bacillus* sp. CECT 40, and *Carnobacterium maltaromaticum* CECT 4134) and two Gram-negative bacteria (*Escherichia coli* B, CECT 101, and *Pseudomonas fluorescens* CECT 378). These species were supplied by the Spanish Type Culture Collection (CECT) of the University of Valencia. These bacterial strains were selected because they are frequently associated with refrigerated foods, either as indicators of foodborne pathogens or as spoilage microorganisms.

2.7.2. Agar Disc Diffusion Method

The strains were stored in cryovials containing 15% (*v*/*v*) glycerol and maintained at −80 °C until use. For experimental assays, active cultures were prepared in sterile Mueller–Hinton broth (MHB) (Biokar Diagnostics, Beauvais, France) and incubated at the optimal growth temperature for each microorganism until the exponential growth phase was reached. Subsequently, aliquots of each culture were transferred into fresh MHB and incubated overnight under the same optimal growth conditions. Culture purity and cell concentration were verified by plate observation and colony counting. The agar disc diffusion method described by Tepe et al. [22] with some modifications was used to determine the antibacterial capacity of eutectic solvent and the date kernel extracts. Thus, a suspension (100 µL of 10^6–10^8 CFU/mL) of each microorganism was spread on the solid medium plates made with Nutrient Agar II (Oxoid, Basingstoke, Hampshire, UK). Filter

paper discs, 9 mm in diameter (Schlinder & Schuell, Dassel, Germany), were impregnated with 40 µL of natural deep eutectic solvent, which was used as a control or natural deep eutectic solvent containing date kernel extract, and placed on the inoculated plates. These plates were incubated, in the case of *Bacillus* sp. and *C. maltaromaticum*, at 30 °C for 24 h; in the case of *P. fluorescens* at 26 °C for 48 h; and in the case of *E. coli* and *L. innocua* at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in triplicate.

2.7.3. Broth Dilution Method

Fresh bacterial cultures were prepared in sterile nutrient broth and incubated at the optimal growth temperature for each microorganism until reaching the exponential growth phase. The inoculum concentration was adjusted to approximately 10^8–10^9 CFU/mL. Nine mL of sterile MHB were transferred into sterile test tubes, followed by the addition of 1 mL of the bacterial inoculum and 1 mL of the corresponding treatment solution (Natural deep eutectic solvent or Natural deep eutectic solvent containing date kernel extract). Control samples were prepared under identical conditions by replacing the treatment solution with sterile distilled water. The final microbial concentration in the assay system was approximately 10^8–10^9 CFU/mL. Then, the tubes were incubated at the appropriate temperature for each bacterial strain for 24 h or 48 h. After incubation, microbial growth was determined by viable cell counting. Serial decimal dilutions were prepared in sterile MHB, and appropriate dilutions were surface-plated on Nutrient Agar II (Oxoid, Basingstoke, Hampshire, UK) medium. Plates were incubated under suitable conditions, and results were expressed as log CFU/mL. All experiments were performed in triplicate, and the results were reported as mean values ± standard deviation.

2.8. Statistical Analysis and Optimization Modeling

Experimental data from both Box–Behnken designs were analyzed using the software STATISTICA 8.0 (Statsoft Inc., Tulsa, OK, USA). The behavior of the experimental system was modeled using the second-order polynomial Equation (1). The statistical significance of the model and its terms was evaluated by analysis of variance (ANOVA) at a confidence level of 95% ($p < 0.05$). Response surface methodology (RSM) plots were generated from the fitted models to visualize the interactions between the extraction parameters.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=j}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j}^{k} \beta_{ij} X_i X_j \quad (1)$$

where Y represents the dependent (response) variable, X_i and X_j denote the independent variables (coded factor levels), β_o is the intercept, and β_i, β_{ij}, and β_{ij} are the regression coefficients for linear, interaction, and quadratic terms, respectively. The goodness-of-fit and quality of the models were evaluated using the coefficient of determination (R^2), adjusted R^2, the lack-of-fit *p*-value, and the coefficient of variation (CV, %). The statistical significance of the models and their corresponding terms was evaluated by analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$). Response surface methodology (RSM) 3D plots were generated from the fitted models to visualize the interactions between the extraction parameters.

The experimental results derived from the unifactorial optimization of the solvent-to-solid ratio, the extraction efficiency evaluation of S-UAE, and the antioxidant and antimicrobial assays were reported as mean ± SD. For these data, a one-way ANOVA followed by a post hoc Tukey's test was carried out to determine statistically significant differences ($p < 0.05$) using Statgraphics version 18 software (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results

3.1. Sequential Optimization

Natural Deep Eutectic Solvents (NADESs) have proven to be highly efficient for extracting polyphenols from various plant sources [13,23,24]. Their unique properties depend fundamentally on the hydrogen-bonding interactions between hydrogen donors and acceptors. However, pure NADESs often exhibit high viscosity and density, which restrict mass transfer, limit handleability, and compromise extraction yields [25,26]. Although preparing hydrated or aqueous NADESs can mitigate these drawbacks, it frequently alters or diminishes the solvent's original physicochemical properties and their applications [25]. Another major challenge in utilizing NADESs as extractants is their recovery, recyclability and the subsequent purification of the recovered bioactive compounds [27]. To circumvent this drawback, an effective strategy involves using NADESs directly as carriers of polyphenols for diverse food applications, such as food additives [28] or plasticizers in the development of biodegradable and bioactive films [29,30]. Since this approach eliminates the purification step, the optimization process must focus on maximizing the polyphenol yield per liter of extract. Considering this factor, a sequential optimization method was developed in the present study to achieve the maximum polyphenol content from date kernel in NADESs in the shortest possible time.

3.1.1. Box–Behnken Design with Four Independent Variables

The experimental response values for the four-factor Box–Behnken design are presented in Table 1. The statistical analysis (Tables 2 and S1) demonstrated the high quality of the fitted mathematical models, displaying R^2 and adjusted R^2 values higher than 0.89, alongside non-significant lack-of-fit p-value ($p > 0.05$). Among the four independent variables investigated (NADES concentration, amplitude, specific energy, and solvent-to-solid ratio), only the solvent-to-solid ratio exerted a statistically significant effect on the extraction yield of total polyphenols, flavan-3-ols, and hydroxycinnamic acids. Conversely, for flavonols, the extraction yield was significantly influenced not only by the solvent-to-solid ratio but also by the NADES concentration and the linear interaction between both factors. This result was like those reported by Airouyuwa et al. [13] who reported after a four-factor Box–Behnken design (time, amplitude, NADES concentration and solid-to-solvent ratio), that only solid-to-solvent ratio and NADES concentration were significant factors in the extraction of date seeds.

These findings are closely related to the polarity of the main phenolic compounds extracted under the conditions studied. Our previous studies on date kernels further support these observations. We had earlier quantified the polyphenol content of date kernels and evaluated the free, conjugated, and bound fractions using three conventional extraction methods, observing that this matrix exhibited a high content of soluble compounds [12]. For instance, all glycosylated quercetins and hydroxycinnamic acids were identified in their soluble-free forms. In contrast, flavan-3-ols displayed a homogeneous distribution between free and conjugated forms, with higher amounts of catechin bound in soluble-conjugated structures. As previously noted, the addition of water to NADESs weakens the hydrogen-bonding network among its components, which modifies its physicochemical properties. Dai et al. [25] pointed out that NADESs retain their distinctive supramolecular structure at water dilution levels below 50% (w/w). While an increase in NADES polarity (via water dilution) can favor the extraction of highly hydrophilic compounds like flavan-3-ols and hydroxycinnamic acids, it negatively affects the solubility of quercetin and its glycosylated derivatives, which possess a more hydrophobic character. A similar trend was reported by Dai et al. [25] where increasing the water content in NADES decreased quercetin solubility but enhanced the extraction of carthamin. Likewise, other authors exploring the dilution of

choline chloride–urea NADESs reported that a 60% water dilution was optimal for extracting total phenolic compounds from japonica flower extracts [23]. Furthermore, a significant increase in the extraction of polyphenols—mainly hydroxycinnamic acids such as caffeic acid—was reported from coffee husks when adding 50% water to NADES; notably, the polyphenol concentration remained stable even when water content was further increased up to 60% [31].

Table 2. Second-order polynomial regression coefficients and model validation parameters for the phase I screening design (four-factor Box–Behnken).

	TPC	Flavan-3-Ols	HCA	Flavonols
β0	32.62 (<0.001)	27.79 (<0.001)	2.13 (0.001)	2.47 (<0.001)
Block (1)	7.83 (0.008)	7.18 (0.006)	0.27 (0.085)	0.32 (0.034)
Block (2)	−7.70 (0.008)	−6.86 (0.007)	−0.32 (0.057)	−0.36 (0.025)
β_1	2.29 (0.196)	2.17 (0.155)	0.05 (0.693)	0.06 (0.586)
β_{11}	5.10 (0.068)	4.52 (0.059)	0.24 (0.230)	0.25 (0.147)
β_2	2.56 (0.160)	2.39 (0.128)	0.06 (0.638)	0.09 (0.428)
β_{22}	−2.59 (0.250)	−2.11 (0.260)	−0.17 (0.354)	−0.26 (0.139)
β_3	−0.92 (0.551)	−1.54 (0.272)	0.14 (0.338)	0.49 (0.015)
β_{33}	1.59 (0.448)	1.85 (0.310)	−0.18 (0.347)	−0.13 (0.382)
β_4	−44.74 (<0.001)	−37.53 (<0.001)	−3.21 (<0.001)	−3.52 (<0.001)
β_{44}	30.16 (<0.001)	24.96 (<0.001)	2.33 (0.001)	2.53 (<0.001)
β_{12}	3.70 (0.219)	3.34 (0.191)	0.14 (0.538)	0.15 (0.423)
β_{13}	2.25 (0.415)	1.98 (0.392)	0.17 (0.462)	0.05 (0.766)
β_{14}	−0.56 (0.829)	−1.02 (0.644)	0.24 (0.250)	0.24 (0.250)
β_{23}	2.64 (0.350)	2.36 (0.320)	0.16 (0.499)	0.10 (0.581)
β_{24}	−2.34 (0.400)	−2.17 (0.355)	−0.02 (0.932)	−0.13 (0.505)
β_{34}	−2.40 (0.389)	−1.07 (0.629)	−0.47 (0.110)	−0.77 (0.019)
P_{LoF}	0.085	0.071	0.285	0.098
R^2	0.957	0.953	0.975	0.970
$R^2_{adj.}$	0.905	0.895	0.945	0.934
CV (%)	5.98	5.84	7.98	5.51

β_0 represents the intercept; β_1, β_2, β_3, and β_4, correspond to the linear coefficients for amplitude (X_1; %), specific energy (X_2; J/mL), NADES concentration (X_3, % *w*/*w*), and solvent-to-solid ratio (X_4, mL/g), respectively. β_{11}, β_{22}, β_{33}, and β_{44} represent the quadratic coefficients, while β_{ij} denote the cross-product interaction coefficients. Block (1) and Block (2) represent the orthogonal blocks assigned to isolate nuisance experimental variance. Bold numerical values indicate statistically significant effects ($p < 0.05$) determined via ANOVA using the pure error formulation. TPC: Total (poly)phenolic content; HCA: Hydroxycinnamic acid; $P_{LoF:}$ Lack-of-fit *p*-value; $R^2_{adj.}$: adjusted coefficient of determination; CV: Coefficient of variation.

Considering that flavan-3-ols are the predominant phenolic family in date kernels, the use of diluted NADES successfully minimized solvent consumption, reduced viscosity, and improved overall process handleability. Consequently, while the extraction of the more hydrophilic compounds was predominantly governed by the massive mass-transfer driving force established by the solvent-to-solid ratio, which masked other minor effects such as amplitude (Table 2), flavone recovery was also constrained by thermodynamic solubility thresholds.

The optimal extraction conditions were 55% NADES, 140 J/mL, 75% amplitude, and 1:10 g/mL solid-to-solvent ratio. These conditions were used in a unifactorial optimization with respect to the solid-to-solvent ratio.

3.1.2. Unifactorial Optimization of Solvent-to-Solid Ratio

The solvent-to-solid ratio is typically the most influential variable in the extraction of bioactive compounds from plant matrices [24]. The critical importance of this parameter is directly linked to the mass transfer principles that govern the extraction process; specifically, it dictates the concentration gradient between the plant tissue and the liquid phase, thereby preventing solvent saturation. In line with this, previous research on S-UAE utilizing water

and ethanol (80%, *v*/*v*) as the extraction medium also identified the solvent-to-solid ratio as the most critical factor for recovering polyphenols from date kernels [12].

Figure 1 illustrates the effect of the solvent-to-solid ratio on the concentration of total polyphenols and the three main targeted polyphenolic subfamilies. Broadly, the results demonstrated that a lower solvent-to-solid ratio (higher solute-to-solvent density) resulted in a higher concentration of phenolic compounds in the liquid phase.

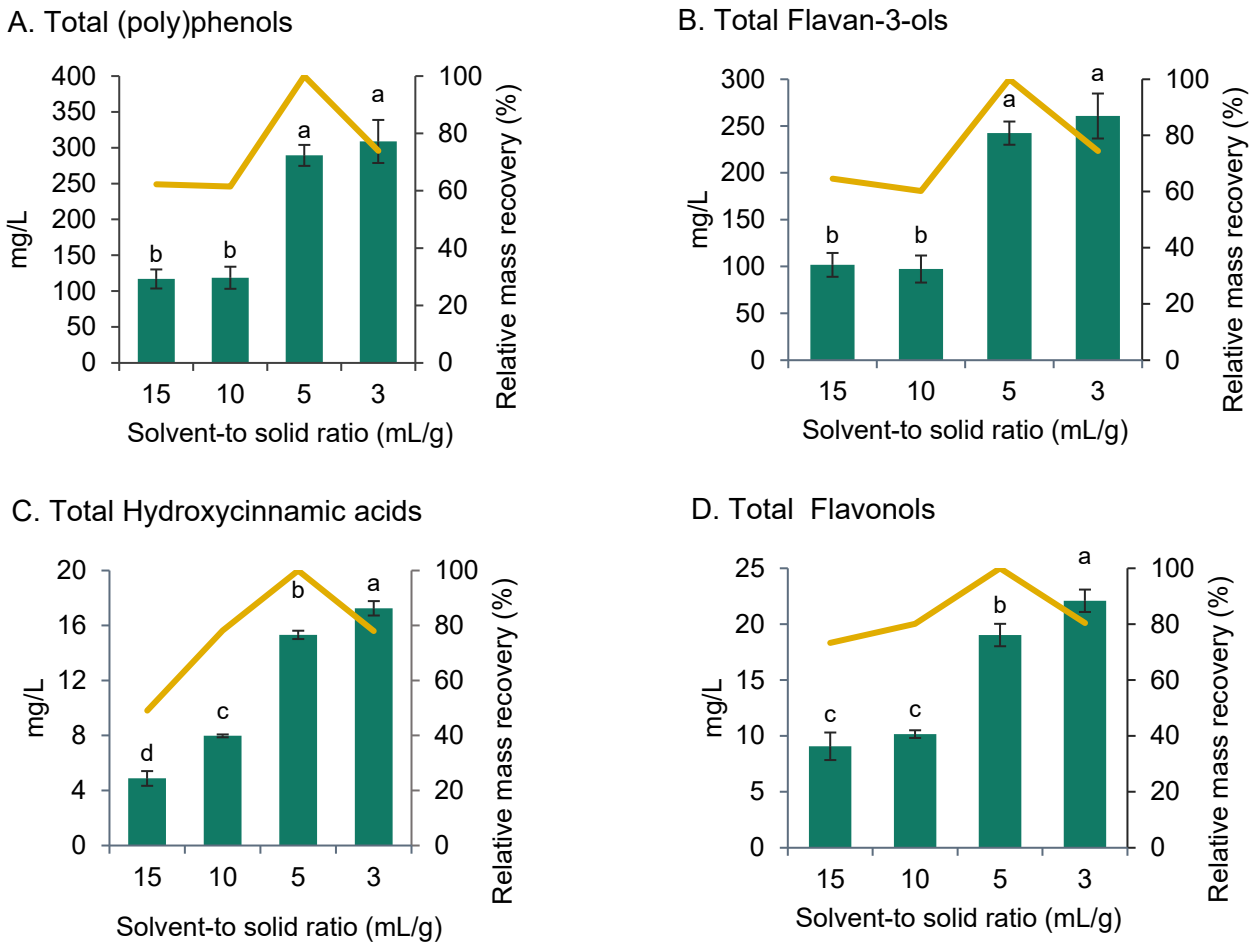

Figure 1. Concentration and relative mass recovery of (**A**) total (poly)phenols, (**B**) total flavan-3-ols, (**C**) total hydroxycinnamic acids, and (**D**) total flavonols from date kernel extracts at different solvent-to-solid ratios tested. Histograms with the same small letter indicate that there are no statistically significant differences ($p > 0.05$) according to Tukey's Multiple Range Test.

Nevertheless, although all polyphenolic subfamilies followed a similar general trend, distinct behavior was observed among them. For instance, the recovery of flavan-3-ols showed no statistically significant differences between the ratios of 3 and 5 mL/g, nor between 10 and 15 mL/g ($p > 0.05$). Similarly, no significant differences were observed in flavonol content when comparing the 10 and 15 mL/g ratios. Conversely, only hydroxycinnamic acids exhibited a progressive and statistically significant increase in concentration as the solvent-to-solid ratio decreased ($p < 0.05$). The results reported in the present work are quite different from those usually reported in scientific papers, where higher solvent-to-solid ratios are related to better phytochemical extractions [32]. However, the aim of the present work is to obtain the maximum recovery of polyphenols per NADES volume, since no purification or concentration process was carried out and NADES was used as a carrier. If we reported the data as mg/100 g date kernel powder, the extraction that generates the best recovery was 15:1 (Supplementary Table S2).

When considering the relative mass recovery, the 5 mL/g solvent-to-solid ratio was selected as the most efficient condition. This variation indicates that the specific chemical nature of each polyphenolic family, along with its cellular distribution within the plant cell wall matrix, strongly determines its mass transfer kinetics into the solvent. Although the 3 mL/g ratio yielded the highest raw concentration values, the relative mass recovery peaked at 5 mL/g (Figure 1). This phenomenon is closely related to the structural swelling capacity of the ground date seeds. At a tighter 3 mL/g ratio, the matrix absorbs a significant portion of the solvent, resulting in a notably lower volume of recoverable supernatant and, consequently, a reduced total mass recovery of target phytochemicals.

3.1.3. Effect of Ultrasonic Variables on NADES Extraction

To comprehensively understand the S-UAE process, three fundamental operational parameters were investigated: amplitude (X_1), specific energy (X_2), and duty cycle (X_3). The ultrasonic amplitude represents the physical displacement of the sonotrode probe tip during each vibration cycle, directly dictating the power density and governing the localized violence of the acoustic cavitation bubble collapse. Meanwhile, the specific energy denotes the actual mechanical and thermal energy delivered per unit of volume to the NADES-matrix mixture. While conventional UAE optimization designs frequently rely on extraction time as a standard variable, substituting it with specific energy provides a monumental advantage for process scalability [33]. Because time is fundamentally non-indicative of the actual energy density, applying identical amplitude and time constraints to a small laboratory vial versus a larger vessel yields drastically different energy dissipation rates, rendering replication impossible. Conversely, specific energy normalizes the energy input against the operating volume, ensuring that the optimized conditions can be precisely replicated during industrial scale-up. Finally, the duty cycle refers to the fractional operating time during pulsed ultrasound applications, where the acoustic wave is intermittently turned on and off. Operating at lower duty cycles effectively helps to prevent uncontrolled temperature spikes and the subsequent thermal degradation of sensitive polyphenols; however, it introduces a significant operational trade-off by substantially increasing the total extraction time required to achieve the target energy input—a drawback that becomes particularly pronounced when combined with low ultrasonic amplitudes.

Table 3 displays the experimental responses obtained from phase II screening (three-factor Box–Behnken). In contrast to the first experimental design, the fitted mathematical models did not exhibit adequate predictive capacity for either hydroxycinnamic acids or flavonols (Supplementary Table S3).

Specifically, the lack-of-fit test for hydroxycinnamic acids was statistically significant ($p < 0.05$), while the coefficient of determination (R^2) for the flavonols model was too low to profoundly explain the variance of the extraction process. This statistical behavior indicates that the specific ultrasonic parameters investigated within these narrower ranges did not significantly influence the extraction kinetics of these two subfamilies of phenolic compounds. In the Phase I design, a subtle quadratic trend for amplitude had been detected for total polyphenols and flavan-3-ols—although it fell just short of statistical significance (p-value 0.068 and 0.059, respectively). Conversely, for hydroxycinnamic acids and flavonols, the dominant driving forces in the Phase I screening design were exclusively the solvent-to-solid ratio and the NADES concentration. Because those two primary variables were already fixed at their optimal values during Phase II, the remaining ultrasound-specific parameters (amplitude, specific energy, and duty cycle) lacked the impact required to establish a significant regression model for these two subfamilies. Consequently, both hydroxycinnamic acids and flavonols were excluded from the final multi-response mathematical optimization modeling.

Table 3. Three-factor Box–Behnken experimental design matrix (expressed in dimensional and dimensionless independent variables) and experimental responses obtained for phase II optimization.

	Independent Variables			*Dependent Variables*			
	A (%)	**SE (J/mL)**	**DC (%)**	**TPC (mg/L)**	**F3O (mg/L)**	**HCA (mg/L)**	**FLV (mg/L)**
No	**X_1**	**X_2**	**X_3**	**Y_1**	**Y_2**	**Y_3**	**Y_4**
1	60 (−1)	60 (−1)	60 (0)	262.40	222.63	10.98	14.64
2	80 (+1)	60 (−1)	60 (0)	316.94	270.84	13.52	16.87
3	60 (−1)	170 (+1)	60 (0)	350.59	306.51	11.54	15.12
4	80 (+1)	170 (+1)	60 (0)	263.92	229.40	9.29	12.22
5	60 (−1)	110 (0)	20 (−1)	185.04	162.67	5.28	7.58
6	80 (+1)	110 (0)	20 (−1)	251.27	216.49	9.63	12.18
7	60 (−1)	110 (0)	100 (+1)	277.47	237.80	11.26	14.16
8	80 (+1)	110 (0)	100 (+1)	261.24	228.44	8.70	11.98
9	70 (0)	60 (−1)	20 (−1)	226.93	195.38	6.75	13.99
10	70 (0)	170 (+1)	20 (−1)	280.58	243.59	9.42	14.86
11	70 (0)	60 (−1)	100 (+1)	262.07	231.66	7.70	9.97
12	70 (0)	170 (+1)	100 (+1)	312.24	271.18	11.27	14.91
13	70 (0)	110 (0)	60 (0)	301.46	264.97	9.40	13.59
14	70 (0)	110 (0)	60 (0)	277.42	243.32	9.01	11.80
15	70 (0)	110 (0)	60 (0)	275.44	238.33	10.03	13.04

X_1: Amplitude (%); X_2: Specific energy (J/mL), X_3: Duty cycle (%). The coded factors are dimensionless and restricted to the [−1, 0, +1] operational space. Experimental responses (Y) are reported as individual measurements for each specific design matrix coordinate. TPC: Total (poly)phenolic content; F3O: Flavan-3-ols; HCA: Hydroxycinnamic acids; FLV: Flavonols.

Among the three ultrasonic parameters studied for NADES extraction from date kernel, amplitude was the least relevant in the extraction (Table 4). Furthermore, to describe the interaction independent variables had with total polyphenols and flavan-3-ols, the 3D surface plots were developed as presented in Figure 2.

Table 4. Second-order polynomial regression coefficients and model validation parameters for the Phase II ultrasound optimization design (three-factor Box–Behnken).

	β_0	β_1	β_{11}	β_2	β_{22}	β_3	β_{33}	β_{12}	β_{13}	β_{23}	P_{LoF}	R^2	$R^2_{adj.}$	CV (%)
TPC	284.77 (<0.001)	2.23 (0.705)	−6.50 (0.479)	17.37 (0.077)	20.20 (0.116)	21.15 (0.054)	−34.51 (0.045)	−35.30 (0.040)	−20.62 (0.104)	−0.87 (0.915)	0.296	0.905	0.734	2.94
F3O	248.87 (<0.001)	1.95 (0.735)	−7.81 (0.400)	16.27 (0.083)	16.29 (0.158)	18.87 (0.064)	−29.71 (0.056)	−31.33 (0.047)	−15.80 (0.155)	−2.17 (0.788)	0.398	0.913	0.756	3.29

TPC: Total phenolic content; F3O: Flavan-3-ols; β_0 represents the intercept; β_1, β_2, and β_3 correspond to the linear coefficients for amplitude (X_1; %), specific energy (X_2; J/mL), and duty cycle (X_3, %), respectively. β_{11}, β_{22}, and β_{33} represent the quadratic coefficients, while β_{ij} denotes the cross-product interaction coefficients. Bold numerical values indicate statistically significant effects ($p < 0.05$) determined via ANOVA using the pure error formulation. PLoF: Lack of fit p-value; $R^2_{adj.}$: Adjusted coefficient of determination; CV: Coefficient of variation.

This statistical profile confirms non-linear, parabolic extraction kinetics regarding the ultrasound pulsation. Initially, increasing the duty cycle provides more effective cavitation time per unit of chronological time, probably accelerating cell wall disruption and mass transfer into the NADES. However, crossing a critical threshold (approaching continuous radiation) triggers a detrimental effect. Operating at excessively high duty cycles suppresses the thermal relaxation periods of the system, leading to rapid, uncontrolled localized temperature spikes. Because flavan-3-ols and co-extracted polyphenols are thermosensitive macromolecules, this continuous thermal and acoustic stress could induce structural degradation, causing the recovery yield to decline after reaching an optimal

intermediate threshold. A Pearson correlation showed that the temperature increase has a positive and significant correlation between both specific energy and pulse (0.63, $p < 0.012$; 0.57, $p < 0.027$, respectively). Therefore, a synchronized balance between the physical force of cavitation (amplitude), energy input, and thermal regulation via duty cycle must be sustained to maximize polyphenol recovery from the *Phoenix dactylifera* L. kernel without risking compound degradation.

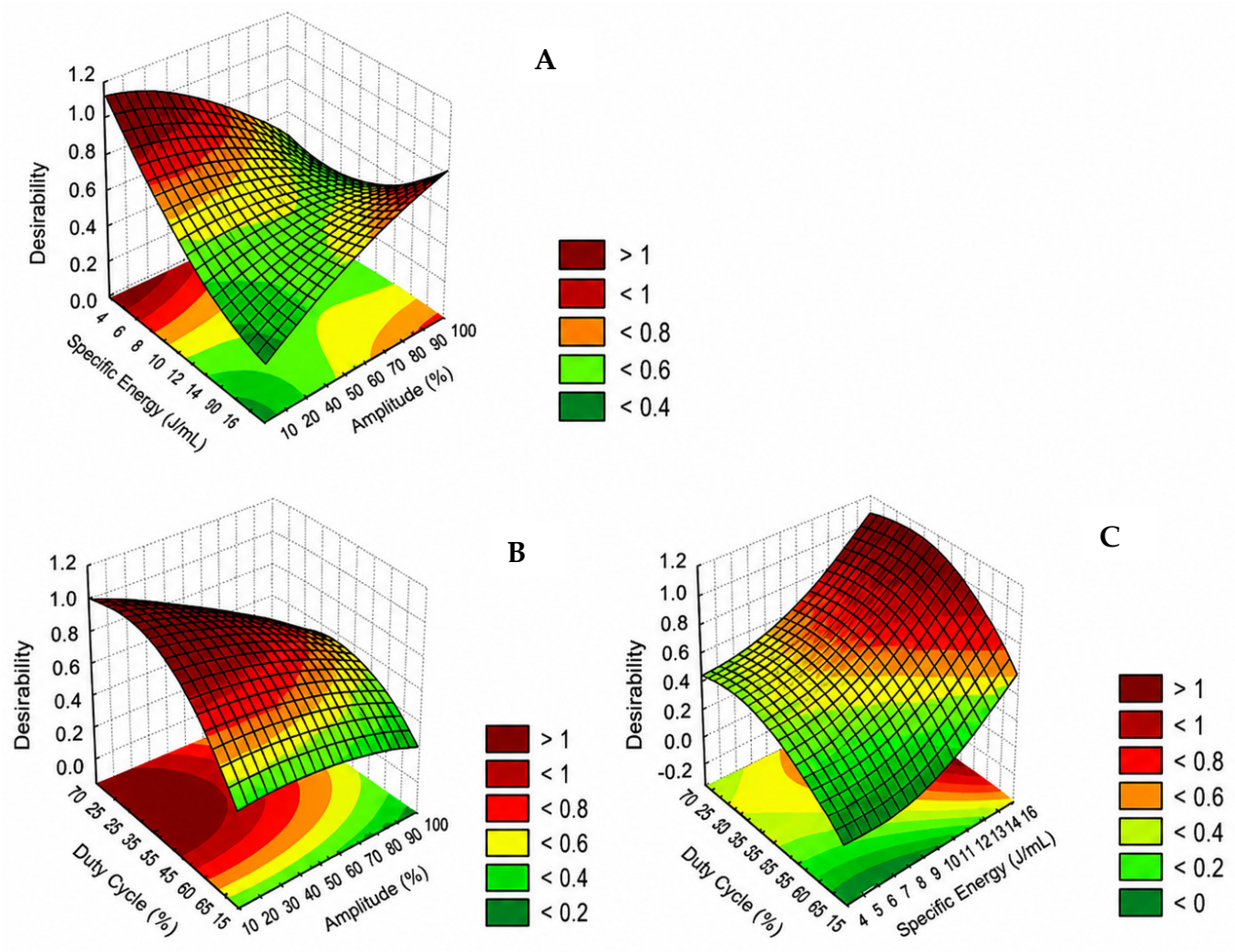

Figure 2. Response surface (3D) plots for the global desirability function, simultaneously optimizing total (poly)phenol content and total flavan-3-ols recovery from date kernel during the phase II optimization design. (**A**) Combined effect of specific energy and amplitude at a fixed duty cycle. (**B**) Combined effect of duty cycle and amplitude at a fixed specific energy. (**C**) Combined effect of duty cycle and specific energy at a fixed amplitude. Coded values on the *Z*-axis represent the normalized desirability function of the target response.

This phenomenon is further modulated by the highly significant negative linear interaction between amplitude and specific energy ($p < 0.05$). The ultrasonic amplitude (X_1) governs the physical displacement of the probe and the violence of the cavitation bubble collapse, while the specific energy dictates the total energy density supplied. The strong negative interaction confirms that applying maximum physical force simultaneously with high energy inputs creates an overly aggressive micro-environment.

3.2. Validation of the Mathematical Model

Table 5 shows the optimal ultrasonic variables to achieve the maximum quantity of total polyphenols and flavan-3-ols from date kernels using NADES diluted with 55% water and at a solvent-to-solid ratio of 5 mL/g. Mathematical equations have also been presented. The coefficient of variation between predicted and actual values was close to 11%. Although this coefficient is typically expected to be below 10% to consider a model predictable [23], given that around 73–75% of the variability can be explained by the model, the moderate predictive ability can be attributed to the variability in fresh NADES preparation (Table 1). Nevertheless, interestingly, under the optimal extraction conditions evaluated (60% amplitude, 170 J/mL, and 80% duty cycle), we achieved the highest total polyphenol and flavan-3-ol concentrations compared to all extractions carried out in this work. Notably, the optimization design successfully obtained the maximum recovery of polyphenols per volume of extract.

Table 5. Optimum process levels, predictive mathematical models, and experimental validation parameters for total polyphenol content and flavan-3-ols for date kernel.

		Optimum Level (Dimensionless)		**Optimum Level (Dimensional)**	
Amplitude (%)		−1		60	
Specific energy (J/mL)		+1		170	
Duty cycle (%)		+0.5		80	
Predictive vs. real response at optimum level					
Response	Predicted	Real	Mean	SD	CV (%)
TPC (mg/L)	360.73	309.78	335.25	36.03	10.75
F3O (mg/L)	311.82	265.81	288.81	32.53	11.27
Mathematical predictive model					
TPC	$Y_1 = 284.77 + 17.37X_2 + 21.15X_3 - 34.51X_3^2 - 35.30X_1X_2$				
F3O	$Y_2 = 248.87 + 16.27X_2 + 18.87X_3 - 29.71X_3^2 - 31.33X_1X_2$				

TPC: Total (poly)phenol content; F3O: Flavan-3-ols; SD: Standard deviation; CV: Coefficient of variation. Real values represent verification experiments (n = 3). In the mathematical models, X_1, X_2, and X_3 correspond to the coded levels of amplitude, specific energy, and duty cycle, respectively.

3.3. Evaluation Efficiency of S-UAE

3.3.1. Polyphenol Profile and Recovery

Considering that NADES possesses an intrinsic capacity to extract soluble compounds per se, evaluating the specific effectiveness of S-UAE required contrasting the ultrasound-assisted extract (DKE-U) against a control extraction where acoustic forces were substituted by conventional rotary agitation (DKE-A). The polyphenolic profile of both date kernel extracts, DKE-U and DKE-A, is presented in Table 6. Both extraction setups yielded an identical qualitative composition. Eighteen polyphenols were detected, of which ten were confirmed by a standard pattern. The profile was quantitatively dominated by the flavan-3-ol subfamily, whereas the flavonols exhibited the highest diversity, with eight distinct compounds identified: the aglycone quercetin and seven glycosylated/methylated quercetin derivatives. This qualitative pattern is consistent with previous characterizations of date kernels [12,34,35].

Table 6. Individual polyphenolic profile of date kernel (mg/L) and antioxidant activity: Comparative evaluation of ultrasound-assisted extract (DKE-U) against a conventional rotary agitation (DKE-A), and percentage increments.

	DKE-U	DKE-A	Increase (%)
Hydroxybenzoic acids			
Protocatechuic acid	9.95 ± 0.97	2.54 ± 0.34 ***	74.23 ± 2.69
Hydroxycinnamic acids			
Caffeic acid	3.14 ± 0.08	1.52 ± 0.46 **	51.5 ± 1.25
5-*O*-Caffeoyl shikimic acid	4.68 ± 0.16	2.39 ± 0.66 **	48.89 ± 1.71
4-*O*-Caffeoyl shikimic acid	6.62 ± 0.24	3.52 ± 0.88 **	46.73 ± 1.91
Flavan-3-ols			
Catechin	47.56 ± 1.15	11.29 ± 1.26 ***	76.25 ± 0.58
Epicatechin	45.91 ± 0.80	17.86 ± 3.88 ***	61.10 ± 0.68
Proanthocyanin 1	55.61 ± 2.10	14.05 ± 2.78 ***	74.71 ± 0.97
Proanthocyanin 2	70.71 ± 1.39	19.67 ± 8.80 ***	72.18 ± 0.54
Proanthocyanin 3	29.62 ± 0.65	7.16 ± 0.62 ***	75.83 ± 0.54
Proanthocyanin 4	16.38 ± 0.64	13.32 ± 1.28 *	20.39 ± 3.34
Flavonols			
Quercetin-3-rutinoside *	1.66 ± 0.07	1.22 ± 0.1 **	26.02 ± 3.24
Quercetin 3-D-galactoside *	2.94 ± 0.05	1.94 ± 0.25 **	33.85 ± 1.05
Quercetin-3-glucoside *	2.30 ± 0.20	1.11 ± 0.04 **	51.21 ± 4.46
Isorhamnetin-3-*O*-glucoside *	4.96 ± 0.12	2.94 ± 0.34 **	40.56 ± 1.42
Quercetin 3-rhamnoside *	2.95 ± 0.02	1.74 ± 0.22 **	40.86 ± 0.4
Quercetin glycoside 6	2.87 ± 0.12	2.20 ± 0.25 *	23.25 ± 3.2
Quercetin glycoside 7	1.42 ± 0.02	1.02 ± 0.09 **	28.13 ± 0.82
Quercetin	0.49 ± 0.02	0.53 ± 0.06	-
Total, (Poly)phenols	309.78 ± 2.24	106.01 ± 20.54 ***	65.78 ± 0.25
Total, Flavan-3-ols	265.81 ± 2.29	83.33 ± 17.17 ***	68.65 ± 0.27
Total, Hydroxycinnamic acids	14.43 ± 0.48	7.43 ± 1.99 **	48.47 ± 1.69
Total, Flavonols	19.59 ± 0.41	12.72 ± 1.12 **	35.05 ± 1.38
DPPH (mg Trolox eq./L)	15.47 ± 0.75	0.97 ± 0.16 ***	92.56 ± 0.31
FRAP (mg Trolox eq./L)	14.70 ± 1.42	1.69 ± 0.31 ***	88.42 ± 1.20

Values are expressed as mean ± standard deviation. DKE-U: Date kernel hydrated natural deep eutectic extract obtained with sonotrode ultrasonic-assisted extraction; DKE-A: Date kernel hydrated natural deep eutectic extract obtained with agitation. * Significant, $p < 0.05$; ** Very significant, $p < 0.01$; *** Extremely significant, $p < 0.001$.

The application of S-UAE significantly enhanced total polyphenol recovery compared to rotary agitation, achieving an approximate 65% increase. The smallest improvements (<50%) were observed for flavonols and hydroxycinnamic acids, which aligns with our Phase II experimental design showing that S-UAE parameters did not significantly influence these subfamilies. Nevertheless, the mechanical action of the sonotrode still provided a substantial recovery improvement in both mentioned phenolic subfamilies, where the three hydroxycinnamic acids were detected, and the main glycosylated quercetin reported significantly high concentrations in DKE-U compared with DKE-S ($p < 0.01$).

The most pronounced enhancement was for flavan-3-ols (close to 70%), with individual compounds such as catechins and proanthocyanidins 1–3 showing highly significant increases ($p < 0.001$) up to 72%. Interestingly, in the DKE-U, protocatechuic acid—a soluble-conjugated or insoluble-bound phenolic acid in date kernels—was successfully detected and quantified. This contrast strongly suggests that the NADES medium facilitates the solubilization of conjugated polyphenols, possibly by promoting the cleavage of ester or ether linkages, more effectively than conventional volatile solvents.

This finding aligns with Airouyuwa et al. [13], who reported that NADESs (e.g., choline chloride-lactic acid) enabled efficient recovery of 3,4-dihydroxybenzoic acid (protocatechuic acid) from date seeds, establishing it as a major phenolic compound in this matrix. Our previous studies on date kernels further support these observations. We had earlier quantified the polyphenol content of date kernels and evaluated the free, conjugated, and

bound fractions using three conventional extraction methods. We also tested food-grade solvents (water, ethanol) in combination with S-UAE. In that prior study, S-UAE with acidified ethanol yielded 37 mg/100 g of free catechins but required a high 200:1 mL/g solvent-to-solid ratio. In contrast, the DKE-U achieves 28 mg/100 g of total catechins with a substantially lower 15:1 mL/g ratio—more than 13-fold less solvent. Although the absolute yield is slightly lower, the dramatic reduction in solvent consumption, together with the ability to recover conjugated catechin forms not accessible with conventional methods, highlights the efficiency of the S-UAE approach. In short, NADES and S-UAE work together to release and stabilize free and conjugated soluble polyphenols from date kernels.

3.3.2. Antioxidant Activity Profile and Recovery

The antioxidant activity of date kernel extracts obtained through different extraction methods showed significant ($p < 0.05$) differences in both DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP), as shown in Table 6. The DPPH assay revealed significant differences in antioxidant activity between the two extracts. DKE-U exhibited an antioxidant activity of 15.47 mg Trolox equivalents (TE)/L, whereas DKE-A showed 0.97 mg TE/L with statistical differences between samples ($p < 0.05$). This indicates that the antioxidant capacity of DKE-U was approximately sixteen times greater than that of the extract obtained by conventional extraction. A similar tendency was observed in the FRAP assay, where DKE-U reached values of 14.7 mg TE/mL ($p < 0.05$) compared to 1.69 mg TE/mL obtained for DKE-A.

The antioxidant activity of date kernel extracts using UAE and DES or conventional solid-liquid extraction procedures is widely demonstrated in the scientific literature. Thus, Airouyuwa et al. [13] reported that the date seeds extracted using UAE and eutectic solvent as an extracting agent had a DPPH value of 719.20 mmol TE/g powder. Pourshoaib et al. [36] found that the antioxidant activity of extract obtained from roasted date seed cultivar Kabkab using UAE was higher than those obtained by maceration with values of 0.97 mg/mL and 0.91 mg/mL, respectively, for DPPH assay and values of 0.55 and 0.30 mg/mL, respectively, in FRAP assay. Zarie et al. [37] informed that the extracts obtained from kernels of three different date cultivars, namely Barhi, Ruthana, and Qatarah, showed important antioxidant activity measured with DPPH and FRAP assays.

These results showed that the UAE-assisted process significantly improved the extraction efficiency of bioactive compounds with antioxidant properties from the plant matrix and enhanced mass transfer and cell wall disruption, facilitating the release of bound phenolic compounds into the solvent [38,39]. In this sense, the results obtained were in concordance with those reported by Mikucka et al. [40] who found that the antioxidant activity, measured with DPPH and FRAP assays, of extracts from distillery stillage obtained using UAE were significantly higher than those obtained using a conventional solid-liquid extraction. Similarly, Aguilar-Villalva et al. [41] informed that the antioxidant capacity of *Annona cherimola* Mill leaves using the UAE was higher compared with the results obtained with conventional extraction techniques.

3.4. Antibacterial Activity

The antibacterial activity of date kernel extract obtained using a eutectic system as a solvent agent was evaluated against five representative food-related bacterial strains, namely *Listeria innocua*, *Bacillus* sp., *Escherichia coli*, *Pseudomonas fluorescens*, and *Carnobacterium maltaromaticum* using two different methodologies: the disc diffusion method and the broth dilution method.

In the case of the disc diffusion method, the application of 40 µL of the eutectic solvent did not produce any inhibitory effect against any of the bacterial strains tested (Table 7). In

contrast, the date kernel extract exhibited antibacterial activity against two of the analyzed microorganisms, with inhibition zones ranging from 12 mm for *P. fluorescens* to 16.00 mm for *Bacillus* sp. Statistically significant differences were observed among the samples ($p < 0.05$).

Table 7. Antibacterial activity of natural deep eutectic solvent (NADES) and natural deep eutectic solvent containing date kernel extract (NADES-KE) using the disc diffusion method.

	L. innocua	*Bacillus* sp.	*E. coli*	*P. fluorescens*	*C. maltaromaticum*
NADES	NA	NA	NA	NA	NA
NADES-KE	NA	16 ± 1^{a}	NA	12 ± 1^{b}	NA

Results are reported as mean ± SD and expressed in mm, including the disc (9 mm); NA: Not active. Values followed by the same letter (^{a,b}) within the same row indicate that there are no statistically significant differences ($p > 0.05$) according to Tukey's Multiple Range Test.

Several studies have previously reported the antimicrobial activity of date kernel extracts using the disc diffusion method. For instance, Gomaa et al. [42] described inhibition zones ranging from 19 to 35 mm for ethanolic extracts of date kernel from the Tamar El Wadi variety at a concentration of 1 mg/mL. In that study, the tested extract produced the largest inhibition zone against *Staphylococcus aureus* ATCC 5638, whereas the smallest inhibition zones were observed against *Salmonella typhi* DSM 17058 and *Shigella sonnei* DSM 5570. Similarly, Osaili et al. [43] reported that date kernel extracts obtained from different date varieties (Fard, Shishi, Lulu, Khalas, Whenetri, Naghal, Bumaan, Barhi, Reziz) at a concentration of 60 mg/mL produced inhibition zones between 17 and 18 mm against *E. coli*, while inhibition against *Listeria monocytogenes* ranged from 20 to 24 mm.

Regarding the broth dilution method (Figure 3), the results revealed significant ($p < 0.05$) differences in microbial susceptibility depending on both the bacterial species and the treatment applied, confirming that the incorporation of date kernel extract substantially enhanced the inhibitory potential of the eutectic formulation.

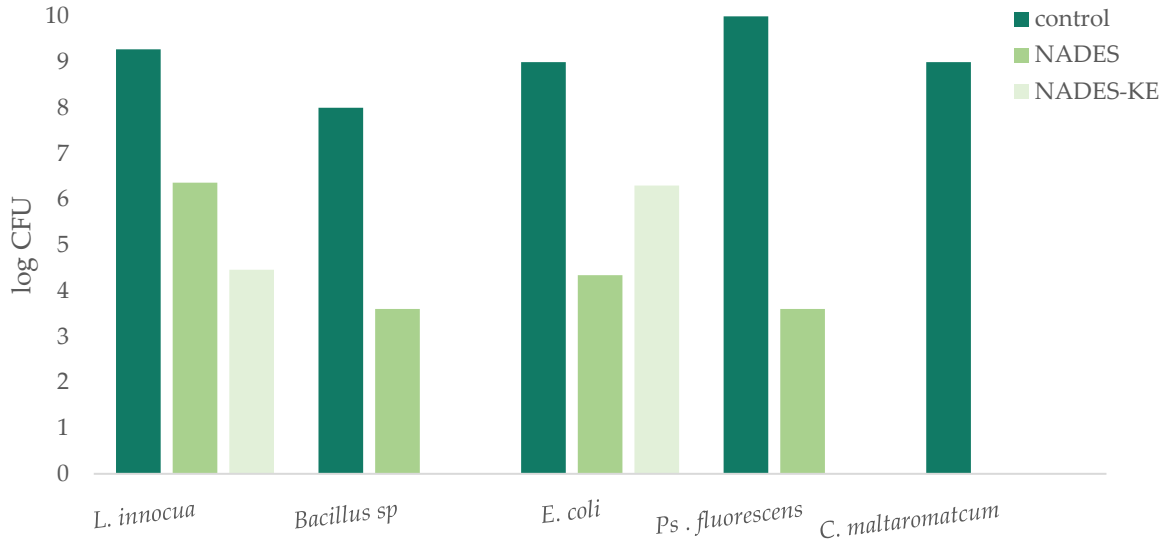

Figure 3. Antibacterial activity of natural deep eutectic solvent (NADES) and natural deep eutectic solvent containing date kernel extract (NADES-KE) using the Broth dilution method.

The control groups exhibited high microbial populations for all tested strains, ranging between approximately 8 and 10 log CFU, indicating unrestricted bacterial growth under the experimental conditions. The eutectic solvent exerted a considerable antimicrobial effect ($p < 0.05$) against all tested bacteria. In comparison with the control, reductions of approximately 2-6 log CFU were observed, depending on the microorganism. The

strongest ($p < 0.05$) inhibitory effect of the eutectic solvent alone was observed against *C. maltaromaticum,* for which complete inhibition of bacterial growth was achieved, with no viable cells recovered after treatment. Similarly, substantial reductions were observed for *Ps. fluorescens* and *Bacillus* sp., whose populations decreased to approximately 3.5 log CFU compared with the control.

On the other hand, *L. innocua* and *E. coli* showed higher resistance to the effect of the eutectic solvent. Although their populations were significantly reduced compared with the control, viable counts remained above 4 log CFU. These results were in agreement with those reported by Ivanović et al. [39], who informed that eutectic solvent containing water, malic acid and sugars like fructose and glucose had antimicrobial effects against several bacterial strains, including *S. aureus*, *Bacillus cereus*, *E. coli*, and *P. aeruginosa.* More recently, Pozharitskaya et al. [26] reported that lactic acid-based eutectic solvent elaborated with choline chloride, sorbitol, or glucose showed antibacterial activities against *E. coli*, *P. aeruginosa*, and *S. aureus* with minimum bactericidal concentrations of 15.6, 7.8, and 1.95 mg/mL, respectively. This behavior suggests that the eutectic system itself possesses intrinsic antimicrobial properties, although the mechanism of action behind this effect is not fully elucidated.

In this sense, Wikene et al. [44] mentioned that the physicochemical characteristics of deep eutectic solvents, including low water activity and low pH, osmotic stress, or chelation of membrane-bound divalent cations, are likely associated with this effect. Additionally, the hydrogen-bonding properties of eutectic systems may facilitate interactions with bacterial cell envelopes, leading to increased membrane permeability and leakage of intracellular constituents [45]. The incorporation of date kernel extract (Figure 3) into the eutectic system further enhanced antimicrobial activity, although the effect observed was strongly dependent on the bacterial strain. Thus, for *Bacillus* sp., *P. fluorescens*, and *C. maltaromaticum*, complete inhibition of cell growth was achieved, while a notable effect was observed against *L. innocua,* whose population decreased from approximately 6.3 log CFU in the eutectic treatment to around 4.4 log CFU after the addition of date kernel extract. This additional reduction suggests an enhanced antimicrobial effect resulting from the combination of the eutectic solvent and the bioactive compounds present in the extract; however, further quantitative analyses would be required to confirm whether this interaction is additive or synergistic. Conversely, in the case of *E. coli*, bacterial counts remained relatively high, reaching values above 6 log CFU, which were even higher than those observed for the eutectic solvent alone.

The antibacterial activity of extracts obtained from date kernels is widely analyzed. Therefore, Hussain et al. [46] reported that the minimum inhibitory concentration (MIC) of date palm kernel extracts obtained from different varieties of *P. dactylifera* L. against different bacterial strains, including *S. aureus* and *E. coli*, ranged from 2.5 to 10 mg/mL. Swaidan et al. [47] found that the date kernel extract obtained from Sakai and Khudari varieties had a MIC value of 41.6 and 20.8 mg/mL for *S. aureus* and *Bacillus cereus*, respectively. More recently, Phasaludeen et al. [48] showed that extracts obtained from date pits using eutectic solvents showed important antibacterial activity, as well as an improved effect of antibiotics against multidrug-resistant *Salmonella typhimurium*.

Date kernel extracts are known to contain considerable amounts of bioactive compounds such as terpenoids, alkaloids and phenolic compounds, including phenolic acids, flavonoids, and tannins [43,49]. In this sense, the antimicrobial properties of phenolic compounds are widely known and are mediated by several mechanisms, including cytoplasmic membrane instability, protein denaturation, metal ion chelation, and interference with enzymatic systems [50,51]. In addition, the eutectic medium may also improve the

solubilization and bioavailability of these phenolic constituents, thereby increasing their interaction with bacterial cells.

4. Conclusions

The present study successfully optimized an extraction process for polyphenols from *Phoenix dactylifera* L. kernel matrix, combining hydrated NADES with sonotrode ultrasonic-assisted extraction. Furthermore, the obtained extract showed antioxidant and antimicrobial activity, with an enhanced antimicrobial effect between NADESs and polyphenols, highlighting the potential of the NADES used as a carrier of polyphenols. The impact of the solvent-to-solid ratio, NADES concentration (%) and interactions among amplitude, specific energy and duty cycle has been proven to govern the extraction of polyphenols present in date kernels, especially the flavan-3-ols, which are the main compounds. Under the optimized conditions, the extracts exhibited antioxidant capacity and antibacterial activity against both Gram-positive and Gram-negative bacteria, supporting the synergistic contribution of NADESs and extracted polyphenols and highlighting the potential of these solvents.

Nevertheless, other parameters probably related to the physical properties of hydrated NADESs remain unexplored. In conclusion, the combination of hydrated NADESs and S-UAE can generate a potentially scalable additive for the food industry while reducing the use of NADES ingredients and lowering extraction time. However, future research should further evaluate the effective recovery of total polyphenols from date kernels, explore scalability, and test their application as antioxidants and antimicrobials in food matrices.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app16146958/s1, Table S1: ANOVA results of Phase I screening design (four-factor Box–Bhenken); Table S2: Concentration of total (poly)phenols, total flavan-3-ols, total hydroxycinnamic acids, and total flavonols from date kernel extracts at different solvent-to-solid ratios tested, expressed as mg/100 g powder; Table S3: ANOVA results of Phase I screening design (three-factor Box–Bhenken).

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