European Journal of Nutrition & Food Safety

Volume 16, Issue 9, Page 203-210, 2024; Article no.EJNFS.122174
ISSN: 2347-5641

Comprehensive Study on Ultrasound-assisted Extraction (UAE) of Bioactive Compounds with Medicinal Properties from Date Processing Byproduct

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ejnfs/2024/v16i91539

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122174

Original Research Article

Received: 28/06/2024
Accepted: 01/09/2024
Published: 09/09/2024

ABSTRACT

The date palm (*Phoenix dactylifera* L.), originating from Mesopotamia, is a crucial fruit crop with significant economic value across the Middle East, South Asia, North Africa, and Central America. Although the edible flesh of dates is prized for its nutritional properties, substantial amounts of byproducts, such as seeds and press cake, are discarded during processing. These byproducts are rich in bioactive compounds, including phenolics, flavonoids, and antioxidants, which have potential health benefits. Conventional extraction methods for these compounds often involve toxic solvents and can degrade heat-sensitive substances. This study explores Ultrasound-Assisted

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Cite as: *K. K, Faheema, Avani Mohan, and Fahmida Komathkandy. 2024. "Comprehensive Study on Ultrasound-Assisted Extraction (UAE) of Bioactive Compounds With Medicinal Properties from Date Processing Byproduct". European Journal of Nutrition & Food Safety 16 (9):203-10. https://doi.org/10.9734/ejnfs/2024/v16i91539.*

Extraction (UAE) as a sustainable alternative for recovering bioactive compounds from date press cake (DPC). The efficacy of UAE was evaluated under various conditions to optimize the yield and quality of the extracted compounds. The study found that UAE significantly enhances the extraction of bioactive compounds, with optimal conditions identified as 15 minutes at 40°C with a 60% ethanol solution. The optimized UAE conditions resulted in a yields of 121.73 mg GAE/g for TPC, 446.66 mg QE/g for TFC, and 67.76% for antioxidant activity. The extracted compounds demonstrated significant antioxidant activity, suggesting their potential application in food, pharmaceutical, and cosmetic industries. The findings indicate that DPC, typically considered waste, can be valorized as a rich source of natural antioxidants and bioactive compounds, offering a sustainable approach to utilizing agricultural byproducts.

Keywords: Ultrasound; bioactive compounds; dates; date press cake.

1. INTRODUCTION

The date palm (*Phoenix dactylifera* L.) with its origins traced back to Mesopotamia, holds vital importance as a fruit crop across regions such as the Middle East, South Asia, North Africa, and Central America. It holds significant economic value as a cash crop and stands as one of the most ancient perennial fruit trees, cultivated since antiquity [1]. The date palm belongs to the Arecaceae family and is recognized as the third most significant palm species within the global agricultural sector, following coconut and oil palms [2]. According to FAO 2021 Egypt is the world's largest producers of date fruits with a production rate of 1747714.68 tonnes. While the edible flesh of dates is highly valued for its nutritional and sensory properties, a significant portion of the fruit, including seeds and other byproducts, is often discarded as waste during processing. These waste materials have been found to be rich in various bioactive compounds, such as phenolics, flavonoids, and antioxidants, which possess potential health-promoting properties [3].

Conventional methods for extracting bioactive compounds from plant sources often employ organic solvents. However, these solvents are poisonous, damaging to the environment, and may degrade heat-sensitive substances [4]. Ultrasound-Assisted Extraction (UAE) has recently emerged as an effective and sustainable method for extracting bioactive compounds from a wide range of plant sources, including agricultural and food processing waste [5]. UAE uses high-frequency sound waves to induce cavitation bubbles in the solvent medium, which then collapse, resulting in localised high temperatures and pressures [6]. This phenomenon enhances the mass transfer of target compounds from the solid matrix into the solvent, leading to improved extraction efficiency and reduced processing time compared to conventional techniques [7].

Despite the potential benefits of UAE for the extraction of bioactive compounds from date waste, there is a need for a comprehensive understanding of the effects of various process parameters, such as ultrasonic power, frequency, temperature, and solvent composition, on the yield and quality of the extracted compounds. Additionally, the structural and functional properties of the extracted bioactive compounds, as well as their potential applications in food, pharmaceutical, and cosmetic industries, warrant further investigation.

This research aims to evaluate the efficacy of ultrasound-assisted extraction for recovering bioactive compounds from date waste and to optimize the extraction conditions for maximizing the yield and quality of the target compounds. The study estimate proximate analysis and functional properties of date press cake, which is a byproduct of date processing. The study will also characterize the extracted bioactive compounds.

2. MATERIALS AND METHODS

2.1 Materials

Fresh date fruits were collected from the agricultural store of Lovely Professional University. Aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), The Folin-Ciocalteu reagent (FCR), along with 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ethanol (C_2H_5OH), were provided by Food Processing Lab, Department of Food Technology and Nutrition, Lovely Professional University. Gallic acid and quercetin (99% pure) were procured from Sigma-Aldrich, India.

2.2 Sample Preparation

The collected date fruits were rinsed with tap water to eliminate any debris, and pits were separated from the fruit. To facilitate grinding, the de-pitted date fruits were soaked in room-temperature water overnight. After soaking, the water was separated from the date fruits. It is then mixed with a blender. Date juice is removed from the blended sample using a muslin cloth. The obtained date press cake (DPC) was dried in a tray dryer at 55°C for 48 hours. The dried samples were ground into powder and stored in an airtight container.

2.3 Physico Chemical Analysis of DPC

The physicochemical analysis of moisture content, ash content, protein, and fat content was determined using the standard procedure of AOAC (2005).

2.4 Experimental Design

Ultrasound assisted extraction of bioactive compounds from date waste was carried out in a ultrasound bath type sonicator. The model was provided with a digital system for extraction time and temperature (adjustable). The specifications of ultrasonic bath used for the experiment are presented in Table 1. Ultrasound treatment was carried out; ultrasound waves were applied with 180 W, 40kHz ultrasonic bath, Model ZX-031S. The pulse duration and pulse interval refer to the “on” time and “off” time of the sonicator. Ethanol was used at different levels of concentration for efficient and non-toxic extraction of bioactive compounds. The experimental parameters selected in the study were shown in Table 2.

2.5 Ultrasound Assisted Extraction (UAE) of DPC

To extract bioactive compounds from DPC, 1 g of sample was mixed with 10 ml of ethanol (50% and 60%) solvent. The mixture was sonicated using a bath type ultrasound system (model ZX-031S, India) with a varying time temperature combination. The extraction processes were performed at sonication temperatures of 35 ◦C, 40 ◦C, and 45 ◦C; duration of 10, 15, and 20 min; constant power of 180 W; and a frequency of 40 kHz. After UAE treatments the samples were centrifuged at 6000 rpm for 15 minutes. The supernatant was filtered and stored at − 18 ◦C until used for further analyses.

Table 1. Specifications of ultrasonic bath

Name	Ultrasonic Cleaner (ultrasonic bath)
Model	ZX-031S
Electric supply	AC220-240 50Hz
Heating power	200W
Frequency	40KHz
Ultrasound power	180W
Timer	0-30min
Full capacity	6.5L
Processor control	Digital Pro
	Programmable time and temperature control with a digital display

Table 2. Ultrasound assisted extraction parameters

Treatments	Time (Min)	Temperature (°C)	Solvent concentration (%)
T1	10	35	50
T2	15	35	60
T3	20	35	50
T4	10	40	60
T5	15	40	60
T6	20	40	60
T7	10	45	50
T8	15	45	60
T9	20	45	50

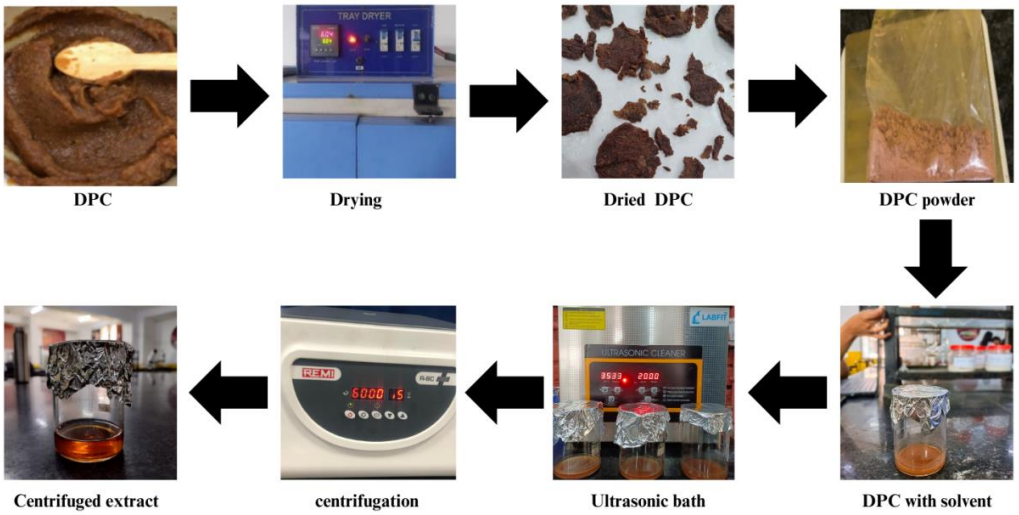

Fig. 1. Ultrasound assisted extraction

2.6 Determination of Total Phenolic Content

The TPC of the date press cake and the date press cake extract obtained through ultrasound-assisted extraction was determined by the Folin-Ciocalteu method with some modifications [8]. A solution was prepared by mixing 0.10 g powdered sample with 3 ml distilled water. 0.5 ml Folin ciocalteau reagent added. Mixed properly for 3 minutes after that 2 mL of Na_2CO_3 (20%) was added and incubated for another 30 minutes at room temperature (22±2ºC) in the dark, a dark blue colour was created with a wavelength of 650 nm. The TPC was quantified using a standard calibration curve prepared with gallic acid, and the results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of sample.

2.7 Determination of Total Flavonoid Content

The TFC of the date press cake extract was determined by the AlCl3 colorimetric method with some modifications [9]. 1 mL of filtered date press cake extracts were diluted with 4 mL of deionized water and mixed thoroughly. Subsequently, 0.3 mL of a sodium nitrite ($NaNO_2$) solution was added, followed by vortexing for 5 minutes. Then, 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added to the mixture, which was vortexed again for 6 minutes. 2 mL of 1 M sodium hydroxide (NaOH) was added and mixed thoroughly. The resulting solution, with a total volume of 10 mL, was stored in a dark environment at room temperature for 30 minutes. The absorbance of the solution was measured at 517 nm using a spectrophotometer. The TFC was quantified using a standard calibration curve prepared with quercetin, and the results were expressed as milligrams of quercetin equivalents (QE) per gram of fresh sample weight.

2.8 Determination of Antioxidant Activity

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) test technique was used to assess the AA of the DPC extract [10]. The absorbance of the samples was measured at 510 nm. The % AA was determined using Eq:

$$A.A\ (\%) = \frac{Ac - As}{Ac} \times 100$$

Where A_c and A_s corresponds to the absorbance of control and sample respectively.

2.9 Statistical Analysis

Results are reported as the mean of 3 replicates ± SD. Statistical significance between groups was analyzed by one-way ANOVA. The significance level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Date Press Cake (DPC)

The proximate analysis of the date press cake (DPC) revealed its compositional profile (Table 3).

The moisture content was found to be 6.1 ± 0.36%, which is within the recommended range for effective storage stability and prevention of microbial, enzymatic, and chemical degradation. This result aligns with previous findings by Marwa A. Sheir [11], who reported a moisture content of 6.11% in DPC. The relatively low moisture content can be attributed to the processing steps involved in date juice extraction, which remove a significant portion of moisture from the fresh dates. The ash content of DPC was determined to be 2.27 ± 0.25%, indicating a moderate mineral content. This result is consistent with the findings of Alqahtani et al. [12], who reported ash content ranging from 2.15 to 2.98% in DPC. The ash content provides valuable information about the overall quality and mineral composition of the product. The fat content of DPC was measured at 1.2 ± 0.1%, which is in line with the results reported by Al-Farsi et al. [13], where fat content ranged from 1.4 ± 0.13 to 2.20 ± 0.11% depending on the date variety. The relatively low fat content in DPC can be explained by the removal of a significant portion of lipids during the juice extraction process. The protein content of DPC was found to be 6.1 ± 0.26%, indicating a moderate protein level. This result is comparable to the findings of Majzoobi et al. [14], who reported protein contents of 6.30 ± 0.35% and 6.41 ± 0.79% in DPC with different particle sizes. The consistency in protein content across studies suggests that DPC could be a potential source of plant-based proteins.

3.2 Bioactive Compounds and Antioxidant Activity

The analysis of bioactive compounds in DPC revealed a total phenolic content (TPC) of 18.62 ± 0.76 mg GAE/g, which is comparable to the findings of Muñoz-Tebar et al. [15], who reported 17.79 mg GAE/g in DPC. The total flavonoid content (TFC) was determined to be 1.89 ± 0.11 mg QE/g, which aligns with the results of Majzoobi et al. (2019). The lower flavonoid content in DPC compared to whole dates can be attributed to the extraction of antioxidants into the date juice during processing.

The antioxidant activity of DPC, measured by DPPH radical scavenging activity, was found to be 35.09 ± 0.17%. This moderate antioxidant activity suggests that despite the extraction process, DPC still retains significant antioxidant properties, making it a potential source of natural antioxidants.

3.3 Effects of Ultrasound-Assisted Extraction (UAE) on Bioactive Compounds

The application of UAE significantly enhanced the extraction of bioactive compounds from DPC. The effects of various UAE parameters (time, temperature, and solvent concentration) on TPC, TFC, and antioxidant activity were investigated (Table 4). Total Phenolic Content (TPC): The highest TPC (121.73 ± 2.92 mg GAE/g) was achieved with treatment T5 (15 min, 40°C, 60% solvent concentration). This represents a 6.5-fold increase compared to the untreated DPC. The results indicate that moderate extraction time, coupled with mid-range temperature and higher solvent concentration, optimizes phenolic compound extraction. This finding is consistent with previous studies showing that temperatures around 40°C are optimal for phenolic extraction without causing significant degradation [16].

Total Flavonoid Content (TFC): The highest TFC (483.7 ± 3.9 mg QE/g) was also observed in treatment T5, representing a remarkable 255-fold increase compared to untreated DPC. This substantial increase suggests that UAE is particularly effective in extracting flavonoids from the plant matrix, possibly due to enhanced cell wall disruption and mass transfer facilitated by cavitation effects [17].

Table 3. Proximate and phytochemical composition of DPC

Moisture (%)	6.1±0.36
Total ash (g)	2.27±0.25
Protein (%)	6.1±0.26
Fat (%)	1.2±0.1
Total phenolic content (mg GAE/g)	18.62±0.76
Total flavonoid content (mg QE/G)	1.89±0.11
Antioxidant content	35.09±0.17

Table 4. Observations Ultrasound-assisted extraction

Treatments	TPC (mg GAE/g)	TFC (mg QE/G)	DPPH Scavenging activity (%)
T1	88.98±0.66^{f}	397±2.31^{h}	59.21±0.41^{e}
T2	103.62±0.76^{d}	408.51±2.79^{g}	58.94±0.35^{e}
T3	104.92±1.52^{cd}	254.07±3.9^{i}	58.11±0.35^{f}
T4	103.77±3.54^{d}	464.07±3.39^{a}	56.82±0.35^{g}
T5	121.73±2.92^{a}	483.7±3.9^{a}	67.76±0.42^{a}
T6	115.58±4.58^{b}	446.66±3.33^{c}	56.46±0.35^{g}
T7	103.84±3.69^{d}	438.88±3.33^{d}	64.27±0.18^{b}
T8	108.63±2.00^{c}	429.62±3.9^{e}	63.29±0.23^{c}
T9	97.97±2.22^{e}	417.77±4.44^{f}	62.31±0.18^{d}

Antioxidant Activity: The DPPH radical scavenging activity showed a positive correlation with TPC and TFC levels. The highest antioxidant activity (67.76 ± 0.42%) was observed in treatment T5, representing a 1.9-fold increase compared to untreated DPC. This enhancement in antioxidant activity can be attributed to the increased extraction of phenolic and flavonoid compounds, which are known for their antioxidant properties.

The results demonstrate that UAE parameters significantly influence the extraction efficiency of bioactive compounds. The combination of ethanol with ultrasound treatment increased the yield of TPC and DPPH radical scavenging activity from DPC.

The optimal conditions (15 min, 40°C, 60% solvent concentration) strike a balance between extraction efficiency and compound stability, as prolonged extraction times or higher temperatures may lead to degradation of heat-sensitive compounds [18].

3.4 FTIR Analysis of Optimized UAE Extract

The FTIR spectral analysis of the optimized UAE extract revealed the presence of various functional groups characteristic of bioactive compounds. The strong absorption bands in the range of 1050-1065 cm^{-1} indicate the presence of C-O bonds typical in phenolic compounds and sugars. The broad absorption in the 3300-2500 cm^{-1} range suggests the presence of carboxylic acids and alcohols, which are common in plant-derived antioxidants. The peaks around 1630 cm^{-1} indicate the presence of conjugated alkenes, which are often found in flavonoids and other phenolic compounds.

The FTIR results provide valuable insights into the chemical composition of the extracted compounds, supporting the quantitative findings of increased TPC and TFC in the UAE extracts. The presence of these functional groups confirms the extraction of various bioactive compounds, including phenolics, flavonoids, and other potential antioxidants.

4. CONCLUSION

This study shows that UAE improves the extraction of bioactive components from date press cake. The solvent concentration had the largest influence on the extraction yield of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity, followed by temperature and extraction time. The best UAE conditions were found to be 15 minutes, 40°C, and 60% solvent concentration, with yields of 121.73 mg GAE/g for TPC, 446.66 mg QE/g for TFC, and 67.76% antioxidant activity. The optimised UAE conditions resulted in substantial increases in TPC, TFC, and antioxidant activity as compared to untreated DPC. These findings imply that DPC, a byproduct of date processing, can be repurposed as a rich source of natural antioxidants and bioactive chemicals. FTIR analysis revealed information about the chemical composition of the isolated chemicals, highlighting the need for additional research to isolate and characterise the specific bioactive components found in the DPC extract. The findings help to promote sustainable resource utilisation by valorising waste streams and recovering high-value components. Future study could concentrate on identifying and characterising certain bioactive chemicals with potential applications in the food, pharmaceutical, and cosmetic industries.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/122174