**Original Research Article**

# Comprehensive study on the extraction of bioactive compounds from date press cake using ultrasound-assisted extraction (UAE)


## 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 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 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.



Key words: 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 (Hussain *et al.*,2020). 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 (Alotaibi *et al.*,2019). 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 (Maqsood *et al.*,2020).

Conventional methods for extracting bioactive compounds from plant materials often involve the use of organic solvents, which can be toxic, environmentally unfriendly, and may result in the degradation of thermally labile compounds (Chemat et al., 2019). In recent years, ultrasound-assisted extraction (UAE) has emerged as a promising alternative for the efficient and sustainable recovery of bioactive compounds from various plant sources, including agricultural and food processing wastes (Chemat et al., 2017). UAE employs high-frequency sound waves to generate cavitation bubbles in the solvent medium, which subsequently collapse, creating localized high temperatures and pressures (Vinatoru, 2001). 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 (Vilkhu et al., 2008).

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 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.LOBA chemie PVT. LTD. provided aluminium chloride ($AlCl_3$) (98% pure), sodium nitrite ($NaNO_2$) (98% pure), sodium hydroxide (NaOH) (97% pure), sodium carbonate ($Na_2CO_3$) (99.5% pure). Gallic acid (98% pure) and Quercetin (99% pure) were obtained from Sigma-Aldrich in India. M/s. LOBA chemie Pvt. Ltd., India, provided the Folin Ciocalteu reagent (FCR), and 2,2-diphenyl-1-picreylhydrazyl (DPPH) (98% pure), ethanol ($C_2H_5OH$) (95% (pure). Distilled water (DI) was obtained from Lovely professional university.

***2.2 Sample preparation***

Date press cake (DPC) were collected after juicing of dates. The press cake was dried, ground into a fine powder using a laboratory mill and stored in airtight containers at room temperature until further use.

***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 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.

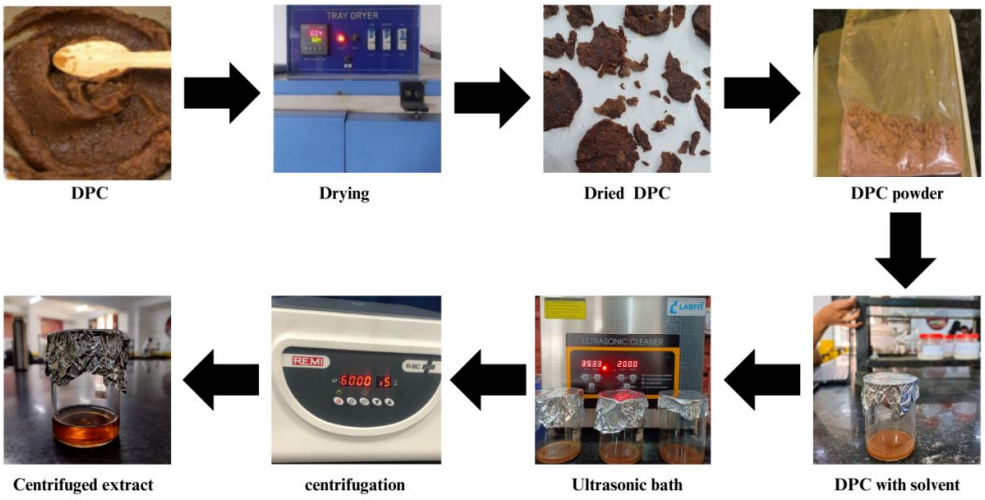


**Fig 1. ultrasound assisted extraction**



### *2.5 Determiation 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 (Kushwaha and Verma 2017). 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.

### *2.6 Determination of total flavonoid content*

The TFC of the date press cake extract was determined by the AlCl3 colorimetric method with some modifications (Tharasena& Lawan 2014). 1 mL of filtered date press cake extracts were diluted with 4 mL of deionized (DI) water and mixed thoroughly. Subsequently, 0.3 mL of a sodium nitrite (NaNO2) 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. Next, 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.

### *2.7 Determination of antioxidant activity*

The AA of DPC extract was evaluated by using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay method (Wani & Uppaluri, 2022). The samples were analysed for their absorbance at 510 nm. Thereby, the % AA was determined using Eq:

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

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

### *2.8 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 1). 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 (2022), 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. (2023), 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. (2008), 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. (2020), 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.

| | |
|---|---|
| **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 1: Proximate and phytochemical composition of DPC.**

***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. (2023), 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.

| Treatments | TPC (mg GAE/g) | TFC (mg QE/G) | DPPH Scavenging activity (%) |
|---|---|---|---|
| T1 | $88.98\pm0.66^{f}$ | $397\pm2.31^{h}$ | $59.21\pm0.41^{e}$ |
| T2 | $103.62\pm0.76^{d}$ | $408.51\pm2.79^{g}$ | $58.94\pm0.35^{e}$ |
| T3 | $104.92\pm1.52^{cd}$ | $254.07\pm3.9^{i}$ | $58.11\pm0.35^{f}$ |
| T4 | $103.77\pm3.54^{d}$ | $464.07\pm3.39^{a}$ | $56.82\pm0.35^{g}$ |
| T5 | $121.73\pm2.92^{a}$ | $483.7\pm3.9^{a}$ | $67.76\pm0.42^{a}$ |
| T6 | $115.58\pm4.58^{b}$ | $446.66\pm3.33^{c}$ | $56.46\pm0.35^{g}$ |
| T7 | $103.84\pm3.69^{d}$ | $438.88\pm3.33^{d}$ | $64.27\pm0.18^{b}$ |
| T8 | $108.63\pm2.00^{c}$ | $429.62\pm3.9^{e}$ | $63.29\pm0.23^{c}$ |
| T9 | $97.97\pm2.22^{e}$ | $417.77\pm4.44^{f}$ | $62.31\pm0.18^{d}$ |

**Table 2: Observations Ultrasound-assisted extraction**

### *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 2).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 (Kumari et al., 2018).

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 (Lafarga et al., 2019).

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 (Benkerrou et al., 2018).

### *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 demonstrates the effectiveness of UAE in enhancing the extraction of bioactive compounds from date press cake. The optimized UAE conditions resulted in significant increases in TPC, TFC, and antioxidant activity compared to untreated DPC. These findings suggest that DPC, a by-product of date processing, can be valorized as a rich source of natural antioxidants and bioactive compounds. The FTIR analysis further supports the presence of these valuable compounds in the extract. Future research could focus on the identification and characterization of specific bioactive compounds and their potential applications in food, pharmaceutical, and cosmetic industries.

**5. Reference**


1. Hussain, M. I., Farooq, M., & Syed, Q. A. (2020). Nutritional and biological characteristics of the date palm fruit (Phoenix dactylifera L.)–A review. *Food Bioscience*, 34, 100509.
2. Alotaibi, M. D., Alshammari, B. A., Saba, N., Alothman, O. Y., Sanjay, M. R., Almutairi, Z., & Jawaid, M. (2019). Characterization of natural fiber obtained from different parts of date palm tree (Phoenix dactylifera L.). International journal of biological macromolecules, 135, 69-76.
3. Maqsood, S., Adiamo, O., Ahmad, M., &Mudgil, P. (2020). Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients. *Food chemistry*, *308*, 125522.
4. Chemat, F., Abert Vian, M., Ravi, H. K., Khadhraoui, B., Hilali, S., Perino, S., & Fabiano Tixier, A. S. (2019). Review of alternative solvents for green extraction

of food and natural products: Panorama, principles, applications and prospects. *Molecules*, *24*(16), 3007.

5. Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry*, *34*, 540-560.
6. Toma, M., Vinatoru, M., Paniwnyk, L., & Mason, T. J. (2001). Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics sonochemistry*, *8*(2), 137-142.
7. Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161-169.
8. Kushwaha, D., & Verma, Y. (2017). Evaluation of antioxidant and free radical scavenging activity of Tagetes patula. *Annual Research & Review in Biology*, *13*(6), 1-8.
9. Tharasena, B., & Lawan, S. (2014). Phenolics, flavonoids and antioxidant activity of vegetables as Thai side dish. *APCBEE procedia*, *8*, 99-104.
10. Wani, K. M., &Uppaluri, R. V. (2022). Recovery of Bioactive Constituents from Unripe Papaya Peel and Pulp Using Ultrasound Assisted Extraction: Optimization of Process Parameters. In *North-East Research Conclave* (pp. 119-139). Singapore: Springer Nature Singapore.
11. Sheir, M. A. (2022). Innovative use of date (Phoenix dactylifera L.) press cake in the food industry.
12. Alqahtani, N. K., Alnemr, T. M., Alsalem, A. K., Alotaibi, M. M., & Mohammed, M. (2023). Experimental Investigation and Modeling for the Influence of Adding Date Press Cake on Drinkable Yogurt Quality. *Foods*, *12*(6), 1219.
13. Al-Farsi, M. A., & Lee, C. Y. (2008). Nutritional and functional properties of dates: a review. Critical reviews in *food science and nutrition*, 48(10), 877-887.
14. Majzoobi, M., Karambakhsh, G., Golmakani, M. T., Mesbahi, G., &Farahnaky, A. (2020). Effects of level and particle size of date fruit press cake on batter rheological properties and physical and nutritional properties of cake. *Journal of Agricultural Science and Technology*, *22*(1), 121-133.
15. Muñoz-Tebar, N., Viuda-Martos, M., Lorenzo, J. M., Fernandez-Lopez, J., & Perez-Alvarez, J. A. (2023). Strategies for the valorization of date fruit and its co-

products: A new ingredient in the development of value-added foods. *Foods*, *12*(7), 1456.

16. Kumari, B., Tiwari, B. K., Hossain, M. B., Brunton, N. P., & Rai, D. K. (2018). Recent advances on application of ultrasound and pulsed electric field technologies in the extraction of bioactives from agro-industrial by-products. *Food and bioprocess technology*, *11*, 223-241.
17. Lafarga, T., Rodríguez-Roque, M. J., Bobo, G., Villaró, S., &Aguiló-Aguayo, I. (2019). Effect of ultrasound processing on the bioaccessibility of phenolic compounds and antioxidant capacity of selected vegetables. *Food Science and Biotechnology*, *28*, 1713-1721.
18. Benkerrou, F., Bachir bey, M., Amrane, M., &Louaileche, H. (2018). Ultrasonic-assisted extraction of total phenolic contents from Phoenix dactylifera and evaluation of antioxidant activity: Statistical optimization of extraction process parameters. *Journal of Food Measurement and Characterization*, *12*, 1910-1916.