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RESEARCH PAPER

Characterization and bioactive potential of epicuticular wax from date fruits

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Abstract

Date fruits (DFs) are lucrative fruits with both nutritional and medicinal benefits. Although DF byproducts have gained interest as potential functional food ingredients, the functional role of DF waxes remains unexplored. Most plant fruits, including DFs,had a lipophilic cuticular layer; and constitutes of epicuticular wax (EPW), which has an important role in respiration losses, mechanical support, fruit softening, and pathogen resistance. In this study, the compositional and antioxidant properties of EPW from Majdool, Khalas, and Fard DFs were investigated. Moreover, thermal transitions of EPW were examined. Results revealed the highest EPW yield in Majdool (16.62 ± 1.68 mg wax/cm^2) and the lowest in Khalas (0.075 ± 0.008 mg wax/cm^2). Ultraviolet–visible spectroscopy showed maximum absorptivity at 250 and 290 nm across all varieties, corresponding to conjugated dienes and trienes, respectively. Fourier transform infrared peaks confirmed the presence of hydroxyl, methylene, and carbonyl groups, including specific domains of phenolic compounds. Khalas and Fard EPWs reported more total phenolic content and scavenging activity than Majdool EPW. The variations were observed in the melting temperatures of EPW, ranging from 60 °C to 85 °C. These findings establish a theoretical foundation for the potential application of DF EPW in food and pharmaceutical industries.

Keywords

Date fruit, epicuticular wax, antioxidants, phenolic compounds

Introduction

The date palm is the most significant crop in various dry areas of the Middle East and North Africa, producing the highly valued date fruit (DF) that is recognized for its rich nutritional profile and numerous health benefits. Global DF production in 2021 reached approximately 9,656,378 tons with one of the top producer from Middle East and North Africa region (FAOSTAT 2021). DF are considered as a fast energy source (due to more than 65% of sugars) with substantial nutritional value (attributed to a notable dietary fiber content of approximately 5.0%–8.5%) (Kamal-Eldin et al. 2020). DFs have important therapeutic implications, particularly in managing conditions such as diabetes and obesity; moreover, they exhibit potent antioxidant effects (Goni et al. 2000; Guillon and Champ 2000; Al-Shahib and Marshall 2003). On the industrial scale, there are very few date fruit products such as date paste and syrups, which is not sufficient for handling the huge amounts of date fruits produced in the Middle East region and thus suffer huge economical losses. Therefore, it is highly important to valorize certain varieties especially lower-quality date fruit in order to enhance its utilization and commercialization.

Similar to other fruit and plant species, DFs are covered by a cuticle and epicuticular wax (EPW). Cuticular waxes serve as a hydrophobic barrier on the plant surface and play vital roles in plant development and environmental stresses (Yeats and Rose 2013). Moreover, they serve as dynamic barriers against extreme drought conditions and microbial attack, influencing important quality parameters (such as fruit surface, texture and hue), postharvest processing efficacy, transportation, and shelf life (Ji et al. 2023). The cuticular wax layer in plants predominantly comprises fatty acids of chain length between 20 and 40 carbon atoms, primarily of aliphatic hydrocarbon chains and derivatives (such as alkanes, aldehydes, alcohols, ketones, and fatty esters). A higher EPW content contributes to reduced water loss (Wang et al. 2014). Nevertheless, components of wax may vary at different growth stages of the fruit. Notably, under abiotic stresses, such as intense climate temperature conditions, fruit cuticles accumulate a substantial percentage of flavonoids (Jaakola 2013). Pensec et al. (2014) investigated changes in triterpenoid content in grape cuticular wax at different stages of ripening of fruit, and Dominguez et al. (2009) found that the increase of bioactive compounds especially flavonoids in tomato cuticles increased the stiffness of cuticular layer during fruit development. However, no study has explored the EPWs obtained from DF. Hence, the current investigation aimed to determine the composition and functional differences of EPWs between the three DF varieties.

Material and methods

Three DF varieties (Majdool, Khalas and Fard) at Tamr stage were procured from a local supermarket in Al Ain, UAE, (Fig. 1) and kept at −20 °C until EPW extraction. All chemicals and reagents used in this study were obtained from Sigma Chemical Company (St. Louis, USA).

DF surface area estimation and wax extraction

The area of DF considered for extraction of cuticular wax was initially calculated. Each DF was depitted, considering the fruit as a rotational ellipsoid. Surface area was calculated using Vernier calipers, employing the formula $S = 1/3\pi (a^2 + 2ab)$, where a represents equatorial diameter and b represents polar diameter (Yang et al. 2021). The wax quantity was expressed as weight per unit area of fruit (mg cm^{-2}). EPW was extracted by immersing cut DF pieces in chloroform (1:2, w/v) for 30 sec. Chloroform extraction was performed twice; all extracted amount was collected, filtered, and then concentrated via rotary vacuum evaporator at 50 °C to obtain EPW (Belding et al. 1998). Wax extraction for each variety was conducted in triplicate.

Characterization of EPW from DF varieties

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy of EPW spread over the KBr tablet was performed using Spectrum Two (PerkinElmer Inc. Shelton, CT, USA). Absorbance was measured in the wavenumber range 4000–400 cm^{-1} using a Mercury Cadmium Telluride—MCT detector and was reported as percentage transmission and wavenumber (cm^{-1}) (Thomas et al. 2022).

Differential scanning calorimetry (DSC)

Samples (10–15 mg) were loaded into hermetic aluminum pans, sealed, and subjected to DSC (TA Instruments Q100, DE, USA), precooled to −80 °C. The empty pan served as the reference, and samples were heated from 10 °C to 200 °C at a ramp rate of 5 °C min^{-1} under a flow of nitrogen gas (40 ml min^{-1}). Using the DSC software, wax melting temperatures (T_m) were determined, and these were plotted with endotherms pointing downward (Bridge et al. 2010).

Ultraviolet–visible spectroscopy

Epicuticular wax (0.1% w/v) was solubilized in hexane and subjected to UV-visible spectroscopic analysis. The solution was scanned at a wavelength of 200–600 nm using a PerkinElmer lambda 25 UV–Vis spectrometer (PerkinElmer, Hopkinton, MA, USA).

Determination of total phenolic content using the Folin–Ciocalteu method

Total phenolic content was determined using the Folin–Ciocalteu method as described by Juhaimi et al. (2012). Wax samples were concentrated to a final volume of 5 ml, and these wax extracts were used for phenolic content

Figure 1. Three varieties of dates.

analysis. The extract mixture was incubated for 30 min at room temperature. Then, absorbance was measured at 765 nm using an using a PerkinElmer lambda 25 UV–Vis spectrometer (PerkinElmer, Hopkinton, MA, USA). The total polyphenolic content was determined against a gallic acid (GA) calibration curve and expressed as gallic acid equivalent (µg GA/100 g sample).

Antioxidant activity via 2,2-diphenyl-picrylhydrazyl method

Free-radical scavenging activity of DF wax was determined using the 2,2-diphenyl-picrylhydrazyl (DPPH) assay (Thomas et al. 2022). Wax samples were prepared by concentrating the final wax extract volume to 5 ml. The absorbance of all the samples was recorded at 515 nm through PerkinElmer lambda 25 UV–Vis spectrometer (PerkinElmer, MA, USA). The radical scavenging activity of EPW samples was expressed in mg/ml as reducing the initial DPPH radical concentration by 50% which is described as inhibition concentration (IC_{50}). The value was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [1 - (A_{sample} / A_{control})] \times 100.$$

Statistical analysis

All experiments were performed in triplicate and the results were expressed as means ± standard deviations. TPC and antioxidants results were compared using one-way ANOVA and Tukey's a,b test with 95% confidence with the SPSS 23.0 software (SPSS Inc., Chicago, IL, USA).

Results and discussion

The plant cuticle, a multifaceted system, is physiologically relevant, primarily in terms of water loss and transport of nutrients and electrolytes within various plant tissues. Cuticular waxes play a vital role as the primary defensive system for plants against external environmental stresses (Chiu et al. 2020). EPWs amount varies among the three DF varieties with highest in Majdool (16.62 ± 1.68 mg wax/cm^2), followed by Fard (0.192 ± 0.027 mg wax/cm^2) and Khalas (0.075 ± 0.008 mg wax/cm^2). Generally, in blueberries and grapes, the amount and conformation of cuticular waxes changed during different stages of development and also because of environmental conditions but remained unchanged during fruit ripening (Casado and Heredia 2001; Chu et al. 2018). FTIR spectroscopy was used to characterize EPWs by identifying functional compounds and their relative amounts in three DF varieties. The FTIR spectra of EPWs from different DF varieties showed several strong absorptivity in the range of 4000–400 cm^{-1} (Fig. 2). Hydroxyl functional group stretching was observed at 3394–3321 cm^{-1} in waxes. Two robust absorption bands at 2900 and 1100 cm^{-1} were attributed to the methylene groups (CH_2), forming the majority of the aliphatic fraction of the waxes. The absorption band at 1706–1717 cm^{-1} was used to measure the C=O bonds of the carbonyl group and esters. Absorptions observed around 1460–1463 cm^{-1} primarily originated from phenolic compounds (Luque et al. 1994). This spectral region can be monitored to characterize different amounts of phenolics in isolated cuticles from various DF varieties. A series of absorption bands at 1042–880 cm^{-1} corresponded to different C-O and methylene groups.

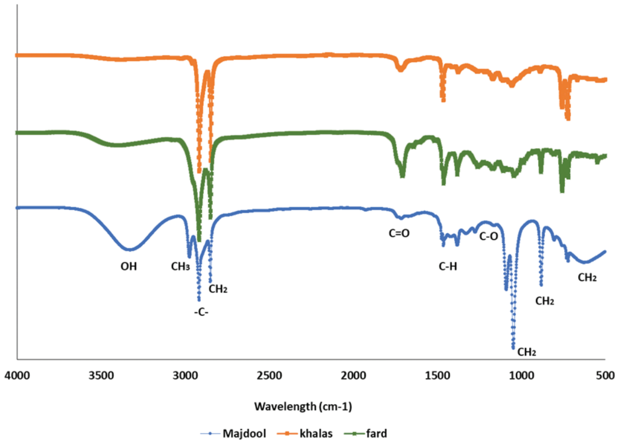

Figure 2. FTIR spectra of epicuticular waxes from different date fruit varieties.

Fig. 3 shows the typical DSC endothermic thermograms of DF cuticles, revealing the melting points of waxes existing in the membrane. The phase transition commenced at around 40 °C in Majdool and Khalas DF, whereas in Fard DF, the transition began around 55 °C. Aliphatic waxes, typically a mixture of compounds varying in chain length, exhibit broad melting ranges instead of distinct melting points (Aggarwal 2001). For Majdool and Khalas, at least 2–3 overlapping peaks indicated the presence of multiple components, whereas Fard predominantly comprised a single component. In Majdool EPW, a first-order transition occurred at 65 °C, and a broad second-order transition extended from approximately 72 °C to 103 °C. The endothermic

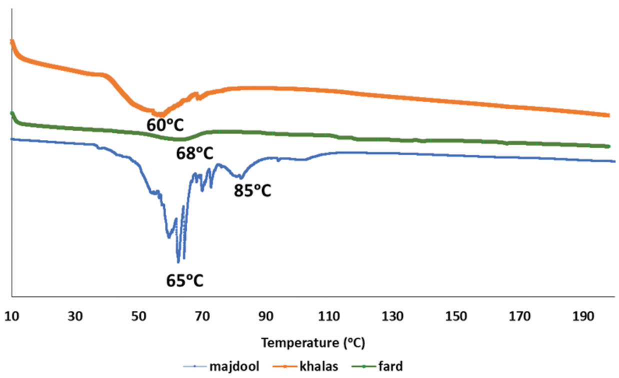

Figure 3. DSC thermogram of epicuticular waxes from different date fruit varieties.

peak at 72 °C could be attributed to the melting of aliphatic chains of wax samples (Casado and Heredia 1999). The presence of substantial amounts of other wax components, is characterized by melting peaks at 65 °C (n-alkanes) or 80 °C–85 °C (long-chain esters). These results align with cuticle behavior, suggesting their crystallinity at physiological temperatures (Casado and Heredia 1999).

The UV–vis spectra of three wax sample types were assessed in the UV range 200–600 nm (Fig. 4). Khalas EPW exhibited higher absorption values than Majdool and Fard EPWs. EPWs showed maximum absorptivity at 250 and 290 nm, corresponding to conjugated dienes and trienes, respectively (Dobarganes and Velasco 2002; Oomah et al. 2006). Lower absorption values observed for Khalas wax samples in the range 390–500 nm may indicate the presence of carotenoid-related pigments (Foppen 1971; Azcan et al. 2000), distinguished by the orange color of the EPW. An absence of absorptivity in the range 535–670 nm indicated the lack of chlorophyll-related compounds in wax samples. Similar findings have been reported regarding wax from flax straw (Athukorala et al. 2009; Yang et al. 2021).

The role of cuticular waxes in different parts of terrestrial plants is protection against environment stresses (Oliveira et al. 2003; Shepherd and Griffiths 2006). In addition to serving as a physical barrier, waxes can act as sources of biologically active compounds influencing food quality maintenance and pathogen susceptibility. Among the three EPW extracts investigated, Khalas EPW showed the maximum total phenolic content, followed by Fard and Majdool EPWs (Fig. 5). A similar pattern was observed for antioxidant activity among the three wax extracts, with peak IC_{50} values of 0.35 ± 0.02 and 0.38 ± 0.04 mg observed for Khalas and Fard EPWs, respectively, indicating their strong potential as free-radical scavengers, whereas Majdool EPW exhibited the lowest IC_{50} value of 0.25 ± 0.02 mg ($P < 0.05$) (Fig. 6).

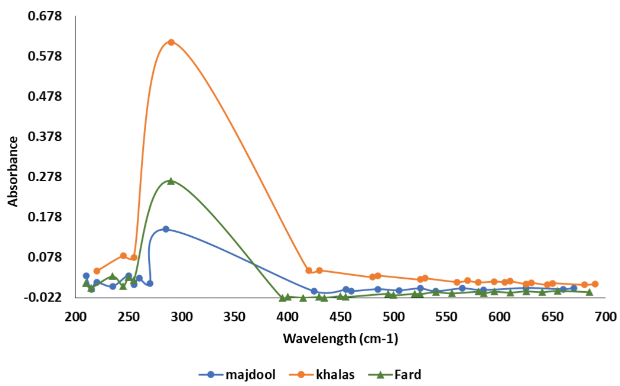

Figure 4. UV-vis spectra of epicuticular waxes from three varieties of date fruit.

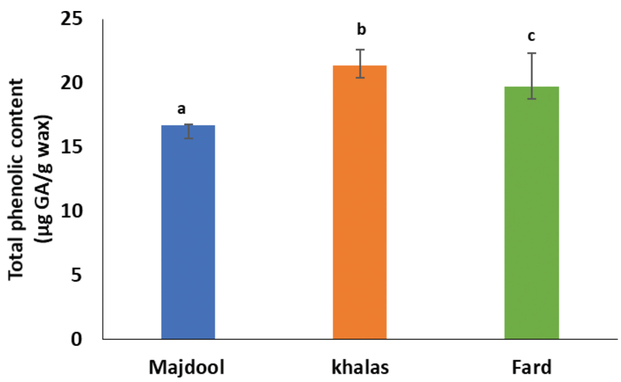

Figure 5. Total phenolic content of epicuticular waxes from different date fruit varieties (n = 3).

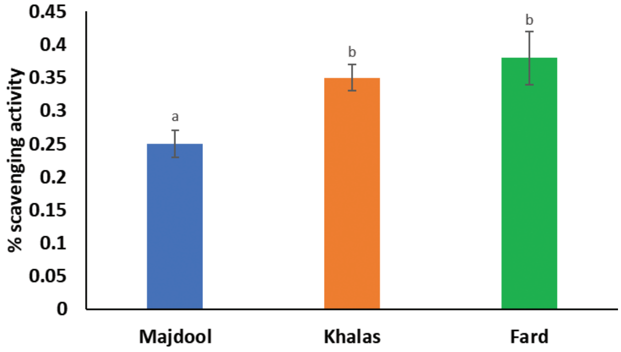

Figure 6. Scavenging activity (%) of epicuticular waxes from different date fruit varieties (n = 3).

Conclusions

In conclusion, the present study confirmed the presence of valuable phenolic compounds in EPWs from three DF cultivars as well as demonstrated the potential radical scavenging activity of these EPWs. High absorption in 250 and 290 nm showed existence of conjugated dienes and trienes. These findings demonstrate the potential application of DF EPWs as natural antioxidants in biomedical, healthcare, food, and agricultural industries as a fruit wax or waxy analogue. However, in this study only the separation of components in the extracts would enable the explanation of the functional compound. The outcomes of this study may serve as a foundation for future research focusing on the functional characterization of the composition and cuticle structure of DF waxes.

Declaration of interest

None.

Declaration of generative Ai in scientific writing

None.

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Author contributions

Bhawna Sobti: Supervision, Writing– original draft preparation, Investigation, Methodology, Project administration, Conceptualization. Khulood Jaber Jasim Alnuaimi, Dema Saeed Bakhit Alneyadi, Osha Obaid Essa Almheiri, Manwa Abdulla Mutaia Alefari: Investigation.

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