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Fingerprinting analysis using crystal based mid-infrared spectroscopy to differentiate between Saudi Dates Fruits for food adulteration

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A R T I C L E I N F O

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A B S T R A C T

Date palm is a commonly edible fruit and has been known widely by the society in the Gulf countries. Dates can be used within the diet of many people due to its contribution in benefits of health and its functions treat various diseases. Date fruits are rich in many valuable elements such as minerals, fatty acids, carbohydrates, vitamins and amino acids. Although there are many varieties of Date fruit, Ajwa Date can be considered to be the most well-known type of dates. In addition, the medicinal and religious value of Ajwa is also known within Muslim populations. Due to the importance value of Dates, it is highly susceptible to adulteration. One of the many forms of adulteration is packing and mixing law quality of Dates for promotion purposes of the known brand to delude the consumers of their high quality. The present study demonstrated that attenuated total reluctance fourier transform infrared (ATR-FT-IR) spectroscopy as a qualitative method for the classification and determination of Date fruit adulteration. Several types of palm Dates (e.g. Ajwa, Rabiah, Sagiel, Meshrg, Khdiry and Safawy) were purchased from local markets in Al Madina Al Monawara. Fruit and seed samples were subjected to ATR-FT-IR spectroscopy for analysis. For discrimination of Date samples, supervise method such as principal components-discriminant function analysis was conducted on raw spectral data. Results exhibited that infrared spectra contained valuable information could allow the differentiation between different types of Dates. Therefore, employing metabolic fingerprinting and supervised chemometric analysis is a proper procedure for food adulteration detects.

1. Introduction

Date palm is a commonly edible fruit and has been known widely by the society of Kingdom of Saudi Arabia (KSA) and other Gulf countries. In KSA, there might be more than five hundred cultivars, and about five thousand species of Date palms grown in various regions globally [1,2]. The production of Dates of several excellent commercial cultivars in KSA is more than one million tons annually. Dates fruit are consumed by large populations in the Middle East and its consumption tends to increase during Ramadan and Al-Hajj seasons. In general, it is consumed either eaten immediately or it might be added to different types of foods and beverages (e.g. pie, cake, jam and juice). Dates can be used within the diet of many people due to its beneficial health and its functions for the protection from poisons according to the statement of prophet Mohammed [3,4]. Although there are many varieties of Date fruit, Ajwa Date can be considered to be the most well-known types of Dates as well as the medicinal and religious value of Ajwa is also known within Muslim populations. In addition, it has been reported that extract of Date fruits is considered as anticancer, antioxidant, antibacterial and antifungal properties and several other competencies in disease protection [5,6]. Date fruits have several nutritional values as it is rich in vitamins, fatty acids, carbohydrates, amino acids and minerals such as magnesium, potassium, calcium, iron and zinc [7-9].

In recent times, food authenticity is growing globally, and the authentication issue in Date fruits is most likely to occur in Date fruit origin. Due to the importance value of Dates, it is highly susceptible to adulteration. Detecting food authenticity for accurate labeling and quality control is the usual respecting religious and economic viewpoint. Therefore, the introduction of convenient analytical approaches for Food authenticity is required. Several analytical methods exist to determine food adulteration, which are mostly based on physicochemical, DNA-based and chromatographic methods [10]. Most of these approaches are expensive, highly time-consuming and require laboratory sample preparations. Recently, chemometric tools coupled with vibrational spectroscopy techniques such as Raman and infrared spectroscopy have been established [11]. Spectroscopic techniques with appropriate chemometric analysis have the ability to investigate multiple objectives employing food fingerprinting [12]. Furthermore, attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy is an inexpensive, non-destructive, simple, short analysis time and accurate methods [13,14]. It is also used to generate information about the composition and molecular structure (fingerprints) of the sample [15]. FT-IR spectroscopy can be used in various fields; for instance, pharmaceutical research [16], food [17,18], agricultural applications [19] and metabolomics [20-22].

2. Results and discussion

FTIR was conducted to evaluate and compare the fingerprinting of seven types of Dates. In order to achieve a better classification of samples, experiments were performed for Date fruits and seeds samples. The FTIR spectra of Date fruit and seed sample is shown in Fig. 1A and B. Several bands in the mid-infrared region between 4000-650 cm-1 were observed which are attributed to a functional group and vibration mode of proteins, lipids and carbohydrate, as Date fruits are rich in proteins, fatty acids, carbohydrates and vitamins. Vibrational assignments of the mid-infrared regions are provided in Table 1[23].

It was clearly noticed that a slight spectral difference among Dates samples was observed as shown in (Fig 1), however, FT-IR spectra are difficult to interpret visually. Thus, a supervised clustering approach such as DFA were performed for data analysis. DFA allows to visualize the samples distribution on the basis of their infrared fingerprint [24]. Therefore, ATR-FTIR data were subjected to PC-DFA in order to generate the first and second discriminant function (DF) scores which allow to identify variation among samples. The resultant DFA scores plots is shown in Fig 2 A and B. it is clearly to be seen that a clear separation among Meshrg, Rabiah, Sagiel, Khdiry and Safawy Date fruits samples is achieved in the first discriminate function that explain the majority of variance among samples. Additionally, similar observations were found the Date seed samples. In addition, it is evident that Ajwa Aliya and Ajwa Madina Date fruits cluster together significantly and separately from other type of Date

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fruits, and also a noticeable slight shift was observed between Ajwa Aliya and Ajwa Madina Date fruits. Although these two types are similar, this variance could be due to harvesting the Date fruits from different palm farms in Al Madina Al-Monawara. Ajwa Aliya Dates fruits are harvested from certain palm farms which was established since the time of prophet Mohammed. Whereas, Ajwa Madina Date fruits is collected from other palm farms in Al-Madina AL-Monawara. This clustering pattern would suggest that different types of Dates fruits can be distinguished from each other due to its fruit origins or nutritional values, indicating the ability of ATR-FTIR coupled with appropriate multivariate analysis as a fingerprinting tool to discriminate between different types of Date fruits and detecting Date fruits adulteration.

To study which spectral regions that caused the discrimination among different types of Dates fruits and Date seeds, the loading plots for PC-DFA and wavenumbers were conducted (Fig 3 A and B). It was clearly observed that some changes take place within these loading plots and the major infrared spectra variances being observed at ν = 2925, 2848, 1555, 1025 cm^{-1}. In addition, it was noticed that the most major infrared spectra variances being observed only ν = 1025 cm^{-1} for Date seeds. These peaks could be associated with chemical groups of components that might present in the Date fruits. The bands at 2925 and 2848 cm^{-1} are responsible for C-H stretching vibrations of fatty acids, whereas these two peaks are not significant and contributing in the separation of Date seed samples. This would suggest that the lipid contents are an important factor to classify Date fruit but not for seed samples. The peaks at 1555 cm^{-1} can be attributed to carbonyl stretching (primary amide) and N-H bending (Secondary amide) vibrations related to the components of protein. Additionally, the band at 1024 cm^{-1} could arise from C-O stretching in the carbohydrate (polysaccharides). Therefore, the noticeable large variations in carbohydrates, proteins and lipids among seven diverse types of Dates could be due to different nutritional contents presents in the Date fruits.

Table 1. Wavenumber and assignment for mid-infrared region**.**

Wavenumber (cm^{-1})	Mode of IR vibration	Vibrational assignment
2960-2850	Asymmetric and symmetric stretches for CH_3 and CH_2 group	lipids
3399-3299	N-H stretching	Amide A of proteins
1691-1619	carbonyl group stretching	proteins (primary amide)
1591-1529	N-H bending and C-N	Proteins (secondary amide)
1450-1200	Carboxyl group of protein	
1200-900	C-O or OH stretching	Carbohydrate

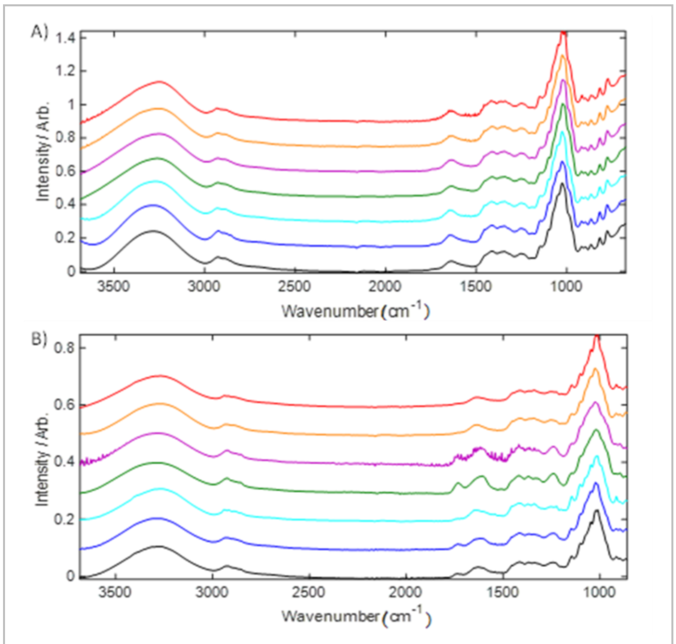

Figure 1. FT-IR spectra collected for (A) date fruits samples and (B) date seed samples. Colours represent different types of dates. Ajwa Aliya (red), Ajwa Madina (orange), Rabiah (pink), Sagiel (green), Meshrg (light blue), Khdiry (dark blue) and Safawy (black).

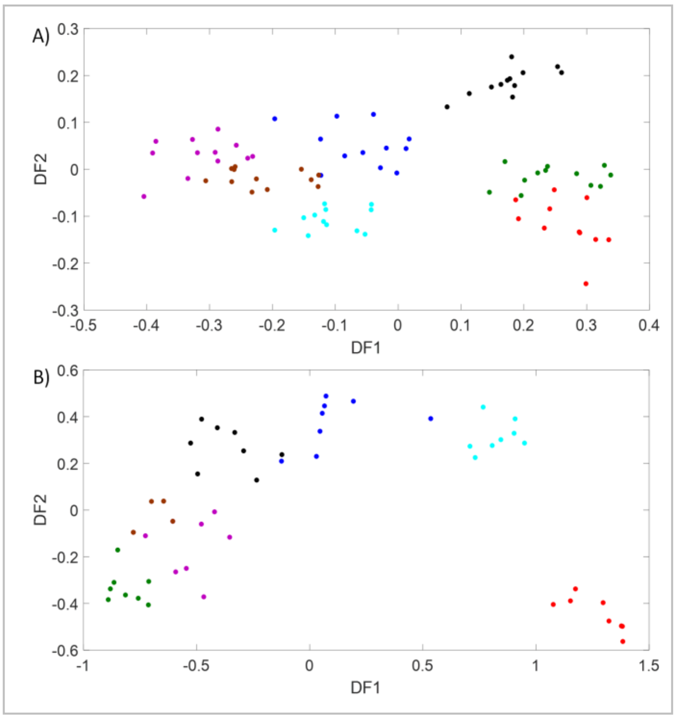

Figure 2. PC-DFA scores plots FT-IR data for (A) date fruits samples and PCs 1-30 with a total explained variance (TEV) of 99.93% were used for the discriminant function analysis (DFA), while (B) date seed samples and PCs 1-21 with TEV of 99.87% were employed for the DFA. Colours represent different types of dates. Ajwa Aliya (red), Ajwa Madina (Green), Rabiah (pink), Sagiel (brown), Meshrg (black), Khdiry (dark blue) and Safawy (light blue).

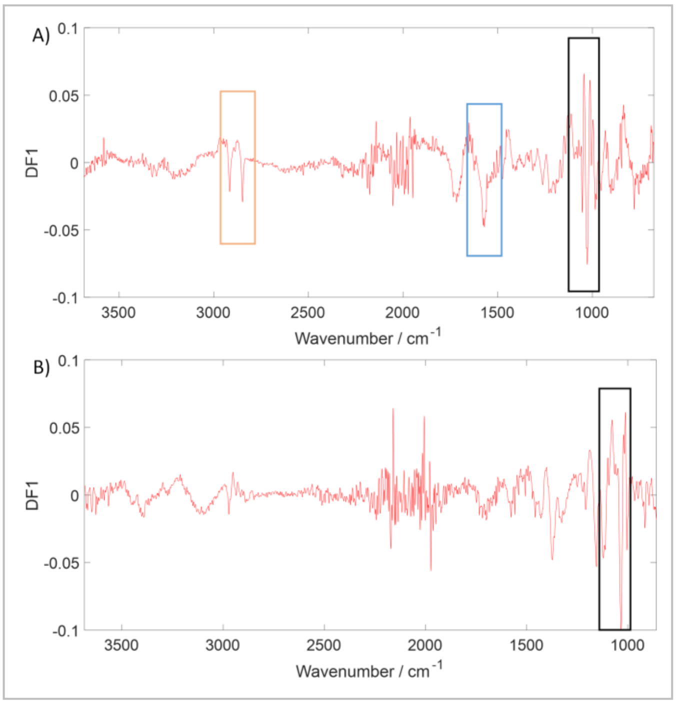

Figure 3. PC-DFA loadings plots from DF1 of (A) date fruits samples and (B) date seed samples. Significant loadings were assigned to carbohydrates, proteins and lipids

3. Conclusions

Our study shows the ability of ATR-FTIR instrument to discriminate between different types of Date samples. Even though many observed bands were similar in Fruit and seed Date samples, their units of absorbance were different because of the variation in the origin and nutritional contents present in the sample. The IR data obtained by ATR-FTIR exhibits that PC-DFA plots demonstrate excellent separations among several types of Dates sample, and the first function (DF1) loading vector demonstrate that several regions derived from carbohydrates, proteins and lipids contribute to this separation.

To sum up, it has been demonstrated that ATR-FTIR technique with a suitable chemometric tool can be a valuable fingerprinting method for classification and detecting Date adulteration according to its origin or nutritional contents.

4. Materials and Methods

4.1 samples collection and preparation for instrumental analysis

Seven types of palm Dates fruits (e.g. Ajwa Aliya, Ajwa Madina, Rabiah, Sagiel, Meshrg, Khdiry and Safawy) were purchased from local Dates central market in Al Madina Al Monawara. Date fruit samples were cut in small round pieces in order to fit the crystal surface. Whereas, Date seed samples were placed on the ATR crystal surface without any modification. Finally, Pressure Clamp was used to press the samples to the crystal surface.

4.2 ATR-FTIR spectra acquisition

Infrared spectra were acquired by using a Niocolet iS50 FTIR spectrometer (Thermo Scientific, Dreiech, Germany) equipped with an ATR crystal (Diamond MIRacle; PIKE Technologies, USA). Measurements were recorded employing a deuterated triglycine sulfate (DTGS) detector from 4000 to 650 cm^{-1} at ambient temperature, with a resolution of 4 cm^{-1}. Finally, 32 scans were co-added and then the mean was calculated in order to enhance signal to-noise ratio (SNR) according to the method proposed by Svecnjak *et al* [25]. All measurements were performed in triplicate for each Date fruit sample, therefore, a total of 84 spectra were collected. The resulting obtained spectra are presented as a graph of absorbance spectra. ATR crystal was carefully cleaned with isopropyl alcohol and then dried prior to analysis.

4.3 spectral pre-processing and data analysis

Initially, raw acquired IR spectra were converted to ASCII files and then imported into Matlab software, version R2016b for operations of multivariate analysis. Prior to analysis, extended multiplicative signal correction (EMSC) were conducted in order to scale the spectra [26].

For cluster analysis, an unsupervised method such as principal component analysis (PCA) was performed on the data for dimension reduction of multivariate data and maintaining the variance [27]. Additionally, a supervised method such as discriminant function analysis (DFA) was then performed in order to separates groups. DFA attempts to increase the differences between the known groups whilst decreasing the differences within the same group [28,29]. PC-DFA was conducted using PCs 1-30 and 1-21 for Date fruits and Date seed respectively.

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Conflicts of Interest: “The author declare no conflict of interest.”

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