Comparative Analysis of Aromatic Properties of Fresh and Dried Date Seed Cinders Using IR and UV Spectroscopy in Accordance with ICH Guidelines

ABSTRACT

Background-Date seeds being a source of edible oil, also used as a functional food ingredient because they are a good source of dietary fiber, phenolic compounds and antioxidants. This research includes study of aromatic property by UV Visible Spectroscopy and Fourier-transform infrared (FTIR) spectroscopy that measures the amount of infrared and ultraviolet light absorbed or transmitted by a sample to obtain spectrum.
The aim of this study is to analyze and differentiate fresh fruit and dried fruit date seed cinders according to ICH guidelines and compare infrared spectra of fresh fruit and dried fruit date seed cinders to identify their distinct profiles.
Result- Quantitative estimation was conducted for moisture, ash, and pH. Both fresh as well as dried date seeds were roasted and then analyzed by IR for the chemical constituents present in them, evaluation of IR spectra and by UV spectroscopy.
Conclusion- Date seeds collected from Dried fruit depicted the highest concentration of distinct constituents. It was analyzed by FTIR study that both the sample does contain constituents of T Butter, Margarine, Lecithin, Olive oil, Sesame Oil, 1- Decanol, n-Nonyl Alcohol, Ethyl Propylene Polymer, Alcohol, Vaseline, Liquid paraffin, Ethyle, Hexadecane, Ethylene Acrylic Acid, 1-Octanol, n- Hexylamine, and Dodecane by UV Spectroscopy it was confirmed that dried date seeds does possess highly aromatic property with aromatic fragrance resembling to coffee which was found to be less in date seeds collected from Fresh fruits.
This research is all about determination of contents in roasted date seeds which can be further used for extraction studies and research development. But the difference was with the olemide, lecithin and some polymers composition in dried date seeds, it was confirmed that dried date seeds does possess highly aromatic property with aromatic fragrance resembling to coffee which was found to be less in date seeds collected from fruits.

Keywords: Fresh Date seeds, Dried Date seeds, Phoenix dactylifra, FTIR, UV-Visible Spectroscopy, Q2, Q14, Aromaticity, Phytochemical test for phenols.

INTRODUCTION

Phoenix dactylifera commonly known as Date Seeds of Genus Phoenix [1]. Date seeds which are essential part of date fruit (Phoenix dactylifera L), with their percentage ranging from 13 to 15% on average of the whole date fruit. Dates processing factories generate thousands of tons of date seeds annually, which are usually ground to feed animals. However, the discarded date seeds from households and restaurants end up in waste bins in, resulting in their underutilization and wastage. Date seeds possess various health benefits, as researchers have found them effective in treating urinary tract infections and fever reduction similar to chemical antipyretics. They also act as immune system stimulants and general body tonics, beneficial for asthma treatment, uterine contraction postpartum, mucous membrane soothing, and certain skin conditions. Additionally, date seeds contain biologically active proteins plays a significant role in cancer detection, with lactins and trypsin inhibitors being among the important proteins.

They are used to strengthen vision, treat hair and scalp conditions. Moreover, date seeds have shown promise in utilizing wasted natural resources. Consequently, alternative approaches have emerged to study the potential use of date seeds as a viable source for consumption. Thus, the study aimed at method development of chemical composition of fresh as well as dried date seeds (Jaipur, Rajasthan) and explore their potential utilization as a Biomedical applicant [2, 3].

Fig No. 1- Fresh Fruit and date seeds

Fig No. 2- Dried Fruit and date seeds

Determination of aromatic property of Date seeds

Aromaticity, which is a chemical property that describes stability and reactivity of a molecule, and is a fundamental concept in chemical structure. Aromatic compounds will always have sweet aromatic fragrance. The chemical quantitative estimations for analysis for the seeds Moisture content determination by measuring the loss in weight of a sample after it's been heated, by utilizing the equation of LOD [4], Ash content estimation of inorganic impurities [5], and pH which measures the acidity or basicity, which is hydrogen ion potential or power of hydrogen [6] [7]. While for the confirmation of aromatic property, Ignition test was utilized that follows Hückel's Rule i.e. a molecule is aromatic if it is cyclic, planar, conjugated and has $4n+2$ π electrons

Phytochemical screening test to confirm aromatic property

Aromaticity of a drug is confirmed by phytochemical tests that can be employed to detect the presence of aromatic rings. Aromatic compounds generally contain conjugated π electron systems, such as benzene rings, which can exhibit specific chemical reactions. Aromatic test for Confirmation of aromatic property in date seeds involves Ferric Chloride Test and Bromine Water Test.

"Whereas Ultraviolet (UV) spectroscopy was used to detect aromatic compounds because they contain a conjugated π electron system. The UV spectrum of an aromatic compound shows a series of bands, with a fairly intense absorption near 205 nm and a less intense absorption in the 255 to 275 nm range. The presence of these bands is a sure indication of an aromatic ring" [39]. Fourier Transform Infrared Spectroscopy (FTIR) technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties.

METHODOLOGY

Drugs and chemicals used

Drug used: Fresh as well as dried date (Jaipur, Rajasthan) were procured from the local vegetable vendor and D Mart (Brand name- Emperor) and then seeds were separated from their fruits and stored at room temperature to dry.

Chemicals: Chemicals and solutions used in the study adhered to high analytical grade standards and were obtained from Loba Chem and Merck Chemical Ltd.

Processing of drug:

Sample Preparation: Date palm fresh fruits were obtained from the local vendor in Jaipur city, ensuring recent harvest as well as dried date fruits were collected from seal packed from local grocery mart. The seeds were first separated from the fruits and then stored under room temperature for 7 days to dry up. Then the seeds were washed with tap water and then underwent process of cleaning with distilled water, drying, and roasting in Hot Air Oven at 100 ± 5 ºC for a period of 48 hours in interval of 8-9 days and then mesocarp of the seed was removed manually, and their weight was recorded to calculate the percentage of adherent mesocarp. Subsequently, the roasted seeds were then pulverized using an electric blender which was further used for analysis.

Chemical Analysis/Quantitative estimation: Various chemical analyses were conducted:

i. Quantitative Estimations: Moisture, ash, pH, conductivity test content were quantified

a) Moisture content- Loss on drying

   Weigh the sample before drying, set the temperature to 100 ± 2ºC, dry the sample, weigh it again, and compare the two weights to calculate the loss on drying.

b) Ash

The ash content is a measure of inorganic impurities in the fuel (typically sand, nickel, aluminium, silicon, sodium, and vanadium), which can cause different kinds of problems. Typically, the ash value is in the range of 0.03%–0.07% by mass.

c) pH

Sample was prepared by mixing 1gm of sample in 1000ml of distilled water and was analysed for its pH by dipping the pH electrode in the solution.

Identification fingerprinting by IR Spectroscopy

Percentage transmittance was studied by IR Spectrophotometer by IR Solution software

1. Selection of mode of spectrum

Firstly select the mode for analysis that is absorbance or transmittance, which will give good peak.

2. Selection of method for sample preparation

3. Selection of wavenumber

Evaluation test

Test For Aromatic Compound Confirmation

1. Ignition test

Put a small amount of the compound on a spatula or porcelain lid and hold it in front of a Bunsen burner flame. If the flame is yellow and sooty, the compound is aromatic.

2. Phytochemical screening test to confirm aromatic property of

Fig No. 3- Filtered extract of Dried and Fresh date seeds

a. Bromine Water Test: Add bromine water dropwise to the drug solution. A positive test is indicated by the disappearance of the orange-brown color of bromine, suggesting the presence of an aromatic ring that has undergone substitution.

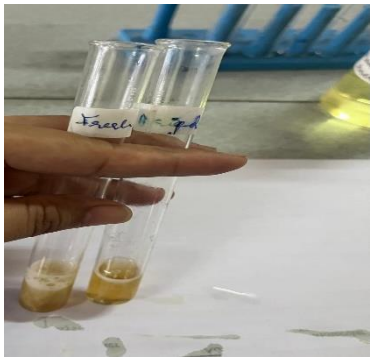


Fig No. 4- Bromine water test Dried and Fresh date seeds

b. Ferric chloride test: About 1 mL of the extract was combined with three drops of $FeCl_3$, and 1 mL of K_2Fe (CN_6). The formation of greenish-blue forms confirmed the presence of phenols.

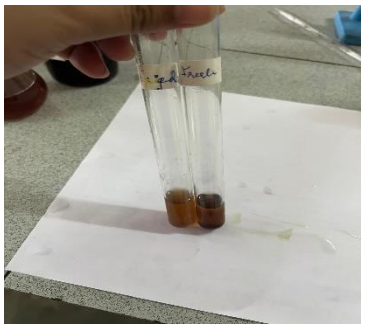


Fig No. 5- Ferric chloride test Dried and Fresh date seeds

2. Ultraviolet (UV) spectroscopy can be used to detect aromatic compounds because they contain a conjugated π electron system. The UV spectrum of an aromatic compound shows a series of bands, with a fairly intense absorption near 205 nm and a less intense absorption in the 255 to 275 nm range. The presence of these bands is a sure indication of an aromatic ring.

RESULT

i. Calculation of Extraction Rate: Analysis of the results presented in Table (1) revealed that the superiority of the fresh fruit seeds in terms of seeds extraction rate, obtaining the highest percentage (30%) of date seeds, with the remaining fleshy part comprising 70%.

Extraction rate served as a determinant of date quality, with higher flesh content indicating superior quality. Conversely, an increase in seeds weight diminished date quality, principle being higher pulp content signified better quality and extraction rate, Flesh, and better seeds percentage.

Table No- 1 Extraction rate

Sample	**% Flesh (Mesocarp)**	**% seed**

Fresh Fruit seeds	40	60
Dried Fruit Seeds	80	20

ii. Moisture content- loss on drying

$$\% \text{ loss on drying} = \frac{\text{Weight loss}}{\text{Weight of sample}} \times 100$$

Table No- 2 % Loss on Drying

Sample	**% Loss on Drying**
Fresh Fruit seeds	67%
Dried Fruit Seeds	57%

iii. Ash value

$$\% \text{ Ash value} = \frac{\text{Ashed weight} - \text{Crucible weight}}{\text{Crucible and sample weight} - \text{Crucible weight}} \times 100$$

Table No- 3 Ash content

Sample	**Ash content**
Fresh Fruit seeds	5.9
Dried Fruit Seeds	4.7

iv. pH

Table No- 4 pH

Sample	**pH**
Fresh Fruit seeds	12.48
Dried Fruit Seeds	13.78

FTIR

Identification fingerprinting by IR Spectroscopy

1. Dried date cinders spectra

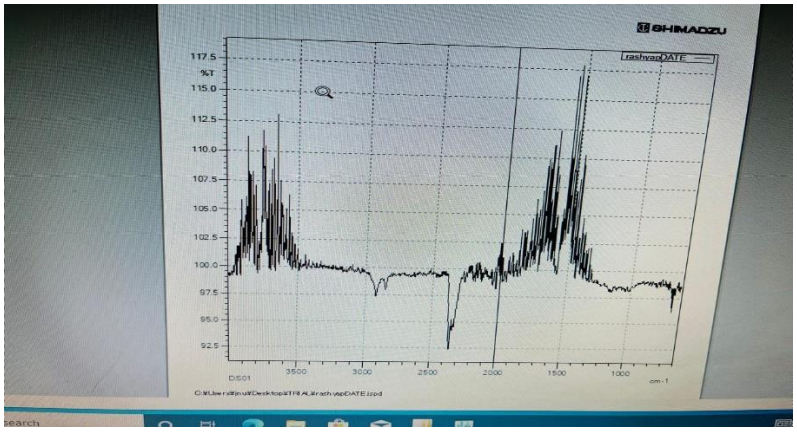


Fig No. 6 – IR Spectra of Dried date seeds

2. Fresh date seeds cinders spectra

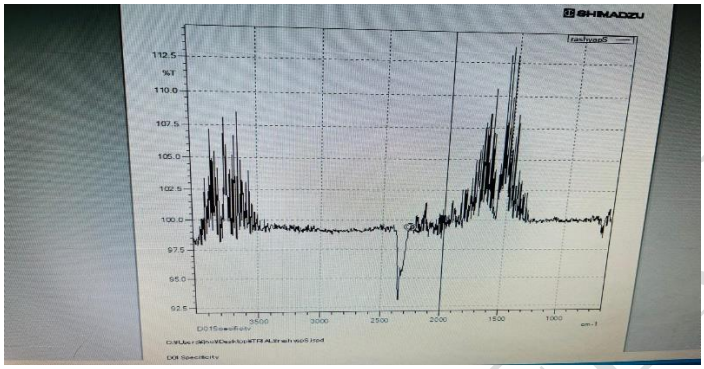


Fig No. 7 – IR Spectra of Fresh date seeds

STEP-BY-STEP ANALYSIS PROCEDURE.

There are five steps to interpret FTIR:

1. Step 1: Identification of number of absorption bands in the entire IR spectrum. If the sample has a simple spectrum (has less than 5 absorption bands, the compounds analyzed are simple organic compounds, small mass molecular weight, or inorganic compounds (such as simple salts). But, if the FTIR spectrum has more than 5 absorption bands, the sample can be a complex molecule, and hence the sample is a complex compound.

Step 2: Identifying single bond area (2500- 4000 cm^{-1}). There are several peaks in this area: (1) A broad absorption band in the range of between 3650 and 3250 cm^{-1}, indicating hydrogen bond. This band confirms the existence of hydrate (H_2O), hydroxyl (- OH), ammonium, or amino. For hydroxyl compound, presence of spectra at frequencies of 1600–1300, 1200–1000 and 800–600 cm-1.

(2) However, the sharp intensity absorption in the absorption areas of 3670 and 3550 cm-1, it

allows the compound to contain an oxygen related group, such as alcohol or phenol (illustrates the absence of hydrogen bonding). A narrow band at above 3000 cm-1, indicating unsaturated compounds or aromatic rings. For example, the presence of absorption in the wavenumber of between 3010 and 3040 cm-1 confirms the existence of simple unsaturated olefinic compounds.

(3) A narrow band at below 3000 cm-1, showing aliphatic compounds. For example, absorption band for long chain linear aliphatic compounds is identified at 2935 and 2860 cm-1 . The bond will be followed by peaks at between 1470 and 720 cm-1 . (4) Specific peak for Aldehyde at between 2700 and 2800 cm-1 .

3. Step 3: Identifying the triple bond region (2000-2500 cm-1) For example, peak at 2200 cm-1 , it should be absorption band of C≡C. The peak is usually followed by the presence of additional spectra at frequencies of 1600– 1300, 1200–1000 and 800–600 cm-1 .

4. Step 4: Identifying the double bond region (1500-2000 cm-1) Double bound can be as carbonyl (C = C), imino (C = N), and azo (N = N) groups. (1) 1850 - 1650 cm-1 for carbonyl compounds

(2) Above 1775 cm-1 , informing active carbonyl groups such as anhydrides, halide acids, or halogenated carbonyl, or ring-carbonyl carbons, such as lactone, or organics carbonate.

(3) Range of between 1750 and 1700 cm1 , describing simple carbonyl compounds such as ketones, aldehydes, esters, or carboxyl. Below 1700 cm-1 , replying amides or carboxylates functional group.

(5) If there is a conjugation with another carbonyl group, the peak intensities for double bond or aromatic compound will be reduced. Therefore, the presence of conjugated functional groups such as aldehydes, ketones, esters, and carboxylic acids can reduce the frequency of carbonyl absorption.

(6) 1670 - 1620 cm-1 for unsaturation bond (double and triple bond). Specifically, the peak at 1650 cm-1 is for double bond carbon or olefinic compounds (C = C). Typical conjugations with other double bond structures such as C = C, C = O orFor the simple aromatic compounds, several bands can be also observed between 2000 and 1700 cm-1 in the form of multiple bands with a weak intensity. It is also support the aromatic ring absorption band (at 1600/1500 cm-1absorption frequency), namely C-H bending vibration with the intensity of medium absorption to strong which sometimes has single or multiple absorption bands found in the area between 850 and 670 cm-1 . 5. Step 5: Identifying the fingerprint region (600-1500 cm-1) This area is typically specific and unique. See detailed information in Table 1. But, several identification can be found:

(1) Between 1000 and 880 cm-1 for multiple band absorption, there are absorption bands at 1650, 3010, and 3040 cm-1 .

(2) For C-H (out-of-plane bending), it should be combined with absorption bands at 1650, 3010, and 3040 cm-1which show characteristics of compound unsaturation.

(3) Regarding vinyl-related compound, about 900 and 990 cm-1 for identifing vinyl terminals (-CH=CH2), between 965 and 960 cm-1 for trans unsatrated vinyl (CH=CH), and about 890 cm-1 for double olefinic bonds in single vinyl (C=CH2).

(4) Regarding aromatic compound, a single and strong absorption band is around 750 cm-1 for orto and 830 cm1 for para, aromatic rings will reduce the intensity frequency with intense or strong absorption bands. When diagnosing unsaturated bonds, it is also necessary to check absorption below 3000 cm-1 . If the absorption band is identified at 3085 and 3025 cm-1 , it is intended for C-H. Normally C-H has absorption above 3000 cm-1 .

(7) Strong intensity at between 1650 and 1600 cm-1 , informing double bonds or aromatic compounds.

(8) Between 1615 and 1495 cm-1 , responding aromatic rings. They appeared as two sets of absorption bands around 1600 and 1500 cm-1 . These aromatic rings usually followed by the existence of weak to moderate absorption in the area of between 3150 and 3000 cm-1 (for C-H stretching).

TEST FOR AROMATIC COMPOUND CONFIRMATION

1. Ignition test

Positive test of ignition.

2. Ultraviolet (UV) spectroscopy can be used to detect aromatic compounds because they contain a conjugated π electron system. The UV spectrum of an aromatic compound shows a series of bands, with a fairly intense absorption near 205 nm and a less intense absorption in the 255 to 275 nm range. The presence of these bands is a sure indication of an aromatic ring.

Table No- 5 Dried date seeds UV Spectral Linearity table at 279nm and 306nm.

Concentration	Absorbance (279nm)	Absorbance (306nm)
10	0.182	0.102
20	0.340	0.145
30	0.509	0.288
40	0.648	0.368

Fig No.- 8 Dried date seeds UV Spectral Linearity graph at 279nm and 306nm.

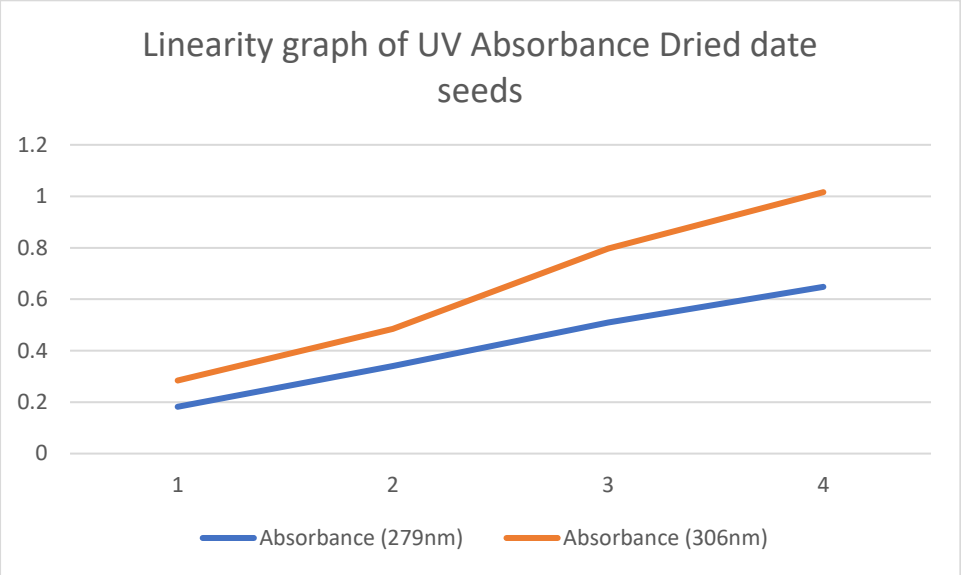
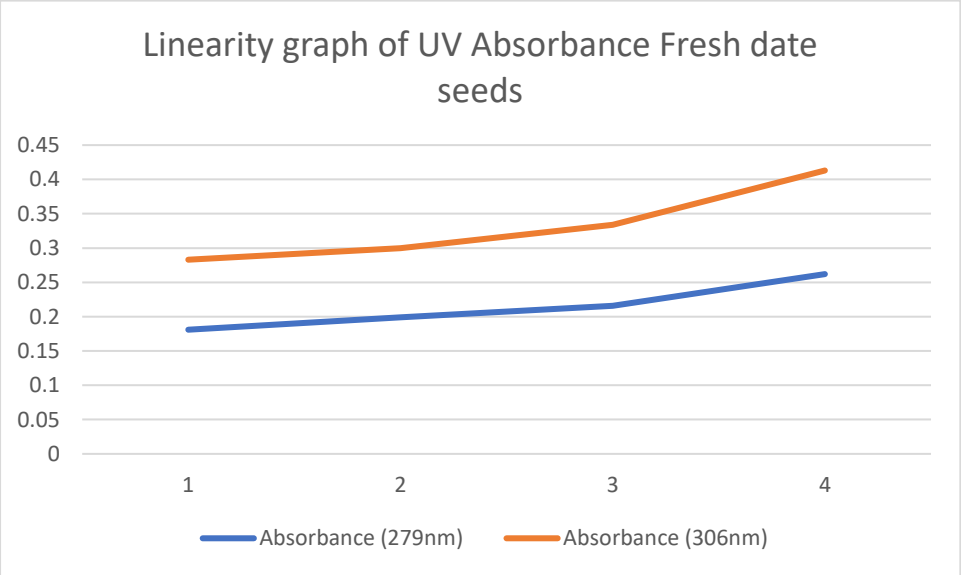


Table No- 6 Fresh date seeds UV Spectral Linearity table at 279nm and 306nm.

Concentration	Absorbance (279nm)	Absorbance (306nm)
10	0.181	0.102
20	0.199	0.101
30	0.216	0.118
40	0.262	0.151

Fig No.- 9 Fresh date seeds UV Spectral Linearity graph at 279nm and 306nm.



IR Spectral pattern of dried dates seeds signifies aromatic property in high amount, fragrance was sweet and resembled like coffee, UV Spectral evaluation was

performed to confirm aromatic functional group in Date seeds, which was more in case of dried seeds and less intense in case of fresh.

SUMMARY & CONCLUSION

In conclusion, this study successfully depicted aromatic behaviour of dried date seeds in high concentration which was also found in case of fresh seeds but in less amount. The aromatic property was validated by performing ignition test and UV Spectroscopic evaluation. A robust IR spectrophotometric method was established, demonstrating the ability to accurately and reliably analyze both fresh and dried date seed cinders, date seed cinders exhibited distinct differences in peak positions, intensities, and overall spectral profiles, allowing for clear differentiation between the two types of cinders. The study provided valuable insights into the chemical composition and structural differences between fresh and dried date seed cinders, as reflected in their IR spectra. The findings of this study have significant implications for the fields of analytical chemistry and food quality control.

Future research could expand on this study by exploring the application of the developed method to other types of seeds or similar materials, further enhancing the understanding of their spectral characteristics. Additionally, investigating the potential of IR spectrophotometry in other analytical contexts could open new avenues for research and application.

In summary, this study demonstrates the effectiveness of using IR spectrophotometry, in compliance with ICH guidelines, to differentiate and analyze fresh and dried date seed cinders, thereby advancing the analytical capabilities in this area and providing a valuable tool for quality control.

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