# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Experimental Design

There are two types of sample in this study; Ajwa dates flesh and human urine. Before the subjects been given Ajwa dates flesh to consume, nutritional compounds of Ajwa dates flesh were determined using Fourier Transform Infrared (FTIR), Gas Chromatography Mass Spectrometry (GC-MS) and Liquid Chromatography Quadrupole-Time of Flight Mass Spectrometry (LC-QToF-MS). These approaches were performed to get the details about chemical compounds of the sample (Ajwa dates flesh). Meanwhile metabolites in human urine were identified using Proton Nuclear Magnetic Resonance ($^{1}$H-NMR), GC-MS and LC-QToF-MS. The design of experiment is illustrated in Figure 3.1.

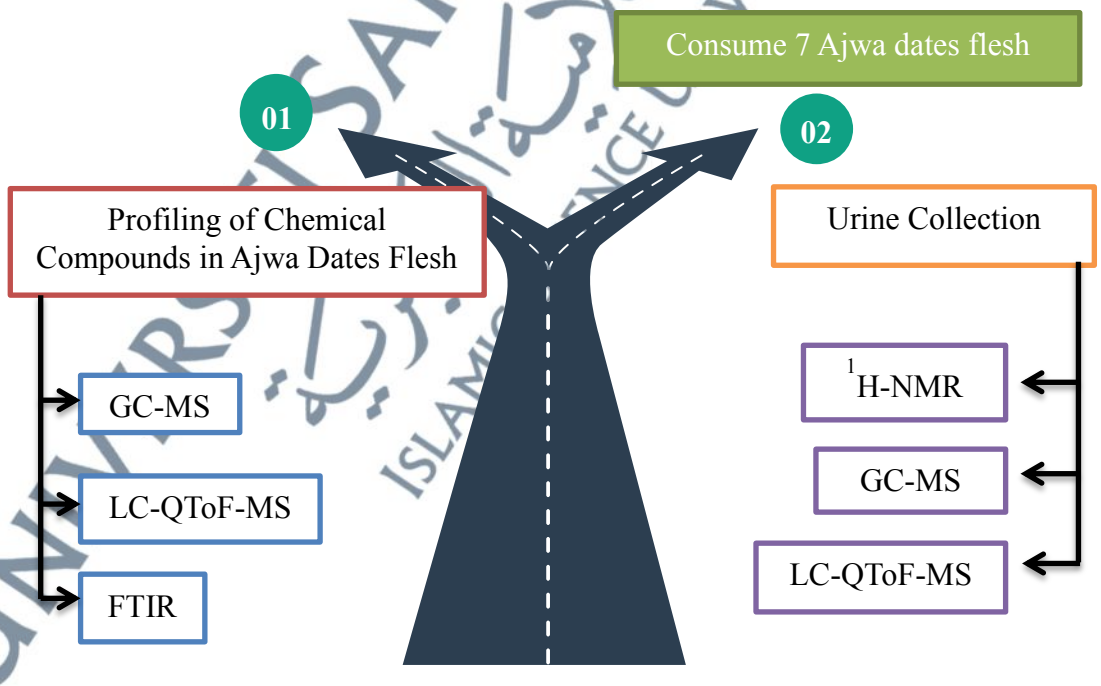



**Figure 3.1:** Summary of Experimental Design

## 3.2 Nutritional Profiling of Ajwa Dates Flesh

### 3.2.1 Chemicals and Materials

Solvents were of GC-MS grade and purchased from Merck (Methanol, Chloroform and Hexane) and Sigma-Aldrich (Supelco-33027: N, O-bis(trimethylsylil) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS)). Ultra-pure water was obtained by purifying of tap water using Ultrapure (Type 1) Water (Direct-Q UV, Merck).

### 3.2.2 Samples Collection

Depitted Ajwa dates (packed in 250 g) batch no (6000/D002, imported from Madinah) was purchased from Syarikat Abdul Ghafar (SAG), Butterworth, Penang. The physical measurement of Ajwa dates is shown in Table 3.1.

Table 3.1: The Physical Measurement of Ajwa dates.

| **Criteria** | **Value** |
|---|---|
| Length (cm) | 3.00 ± 0.141 |
| Width (cm) | 2.38 ± 0.249 |
| Weight (g) | 8.18 ± 1.013 |

### 3.2.3 Sample Preparation

Samples were cleaned and rinsed using distilled water to remove adherent dust particles. The sample was cut uniformly into 5-7 mm size and freshly prepared for extraction. The extra sample was stored at 4 °C until further analysis. Three analyses were conducted on Ajwa dates flesh; FTIR, GC-MS and LC-QToF-MS. All analyses were performed to determine the chemical compounds in Ajwa dates flesh.

### 3.2.3.1 Sample Preparation for GC-MS Analysis

#### 3.2.3.1.1 Preparation of Three Mixture Design Solvents

Three organic solvents namely methanol, chloroform and hexane were mixed to produce mixtures of polar, semi- and non- polar solvents of three mixture designs as illustrated in Figure 3.2.

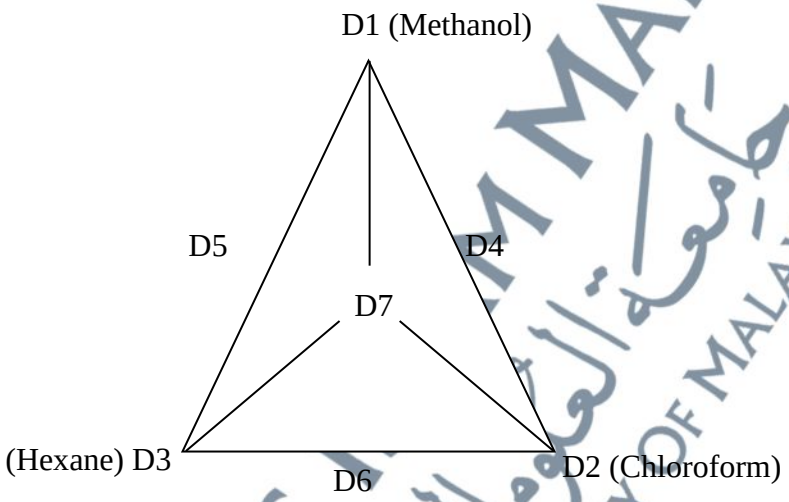



**Figure 3.2:** Three Mixture Design

Based on this concept, seven designs were formed and labelled as D1 (100 % methanol), D2 (100 % chloroform), D3 (100 %, hexane), D4 (methanol: chloroform (1:1)), D5 (methanol: hexane (1:1)), D6 (chloroform: hexane (1:1)) and D7 (methanol: chloroform: hexane (1:1:1)). The combination of methanol and hexane (D5) formed two layers, as both solvents are immiscible. Both layers were collected and labelled as D5M and D5H for the methanol and hexane layer, respectively. The summary of the mixture design are tabulated in Table 3.2.

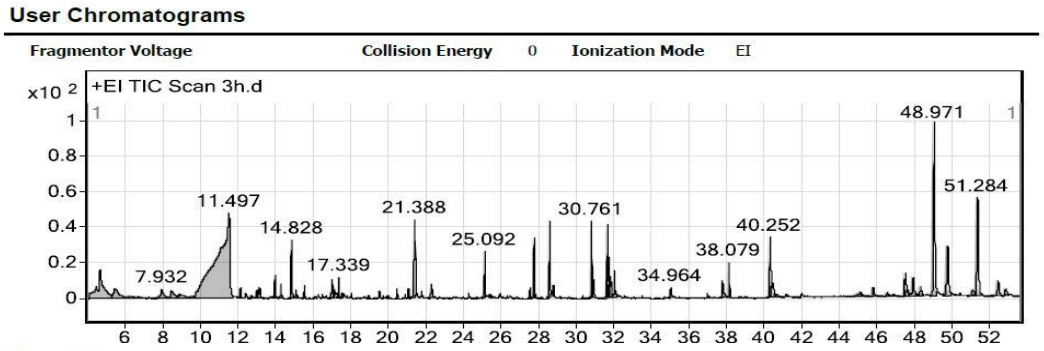
**Table 3.2**: Summary of Mixture Design

| Label | Methanol (mL) | Chloroform (mL) | Hexane (mL) |
|---|---|---|---|
| **D1** | 30 | 0 | 0 |
| **D2** | 0 | 30 | 0 |
| **D3** | 0 | 0 | 30 |
| **D4** | 15 | 15 | 0 |
| **D5** | 15 | 0 | 15 |
| **D6** | 0 | 15 | 15 |
| **D7** | 10 | 10 | 10 |

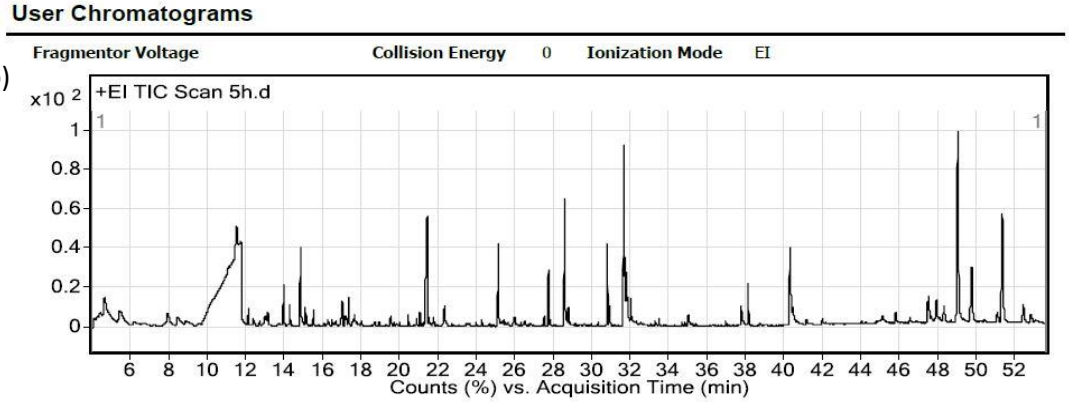
### 3.2.3.1.2 Extraction of Samples

A total of 30 mL solvent was added to one gram (1 g) of dates sample in a 50 mL of conical flask. The extract was left at room temperature for 3 hours. This is based on preliminary study we had done on soaking time extraction (Figure 3.3 a), b) and c)). These three chromatograms showed the same pattern regardless the time soaking (3 hours, 5 hours and 24 hours). Thus, 3 hours for time soaking was chosen based on this qualitative study.

a)




b)



c)

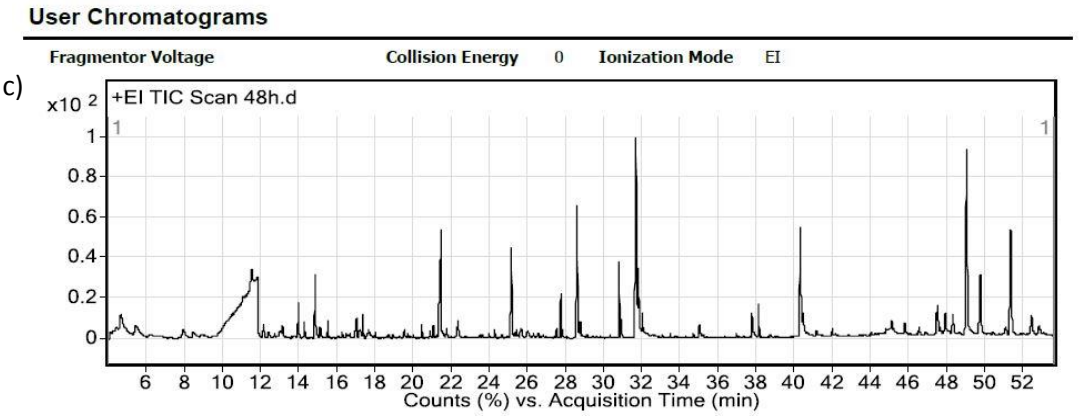



**Figure 3.3**: Chromatogram of Soaking Time at a) 3 hours, b) 5 hours and c) 48 hours.

During the 3 hours soaking time, the extract was vortexed periodically. The extract was then filtered using Whatman No.1 filter paper into a new 50 mL conical flask. Subsequently, the filtrate was concentrated to 1 mL using rotary evaporator at heating bath temperature of 60 °C, followed the 20/40/60 rule (Appendix 1) of rotary evaporator (BUCHI, model R-215, vacuum pump V-700, recirculating chiller F-100 230 V, heating bath B-491, Switzerland). Instead of concentrating the filtrate to dryness, the filtrate was concentrated to 1 mL to maintain the same solvent extraction. Flow of sample extraction for GC-MS analysis is shown in Figure 3.4. This method was referred to Kchaou et al. (2013) with some modifications on the extracting solvent used. They used five different extracting solvents meanwhile in this study we are using three mixtures design. Three independent experiments were conducted in each design.

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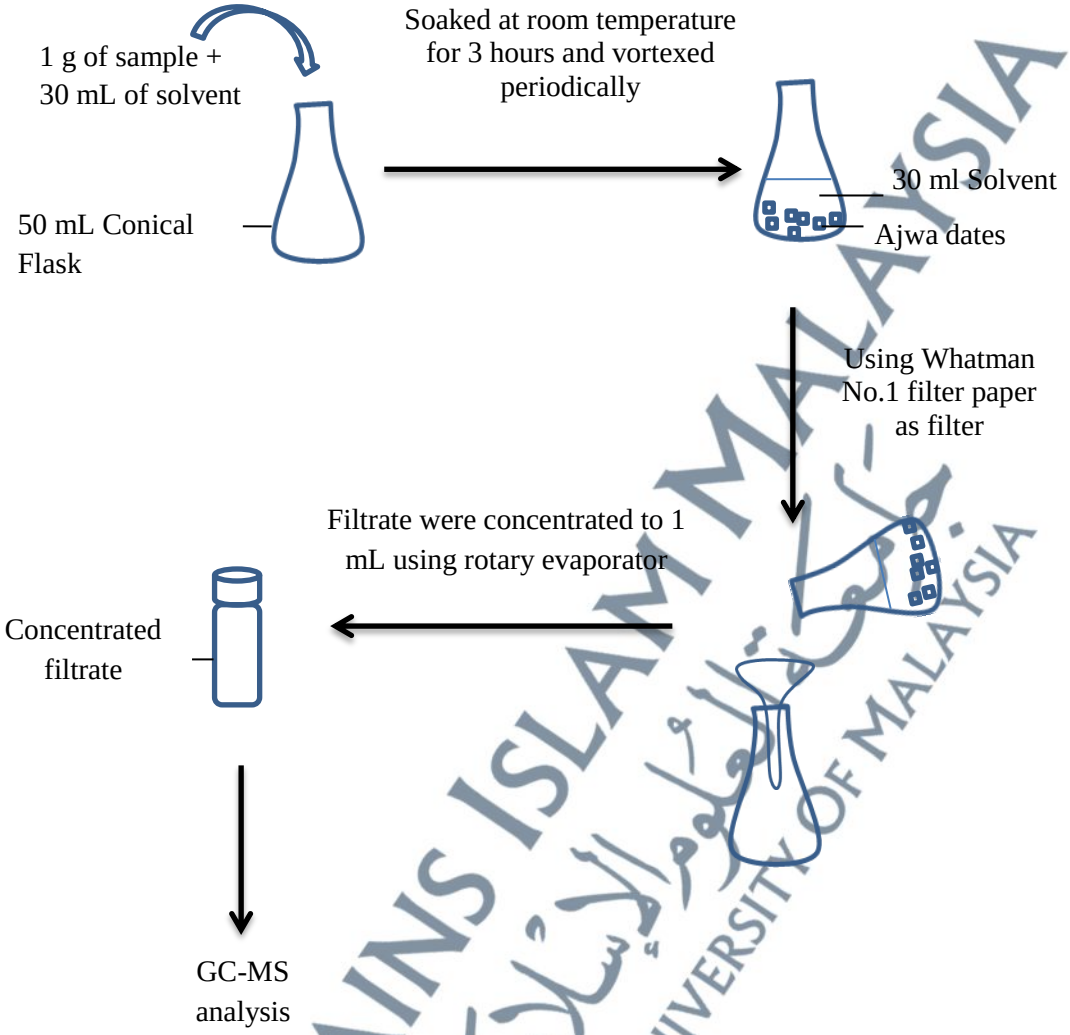


**Figure 3.4:** Flow Chart of Sample Extraction

#### 3.2.3.1.3 Derivatization Process

For derivatization step, which followed the standard procedure of derivatization process with slightly modifications on the heating temperature and time, a total of 80 µL of N, O-Bistrifluoroacetamide (BSTFA) and 20 µL of Trimethylsilyl (TMS) were added to 1000 µL of extract in a 2 mL vial and vortexed to mix thoroughly. The vial was covered with aluminum foil and placed in an oven at 65 °C for an hour. Francis Orata (2012) stated that some studies were heated at 80 °C for 30 min (Szyrwinska et al 2007; Kuo and Ding, 2004) and another study was heated 45 °C for 24 hours

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(Schauer et al., 2002). The samples were later analyzed for chemical compounds using GC-MS. Flow chart of derivatization steps is shown in Figure 3.5.

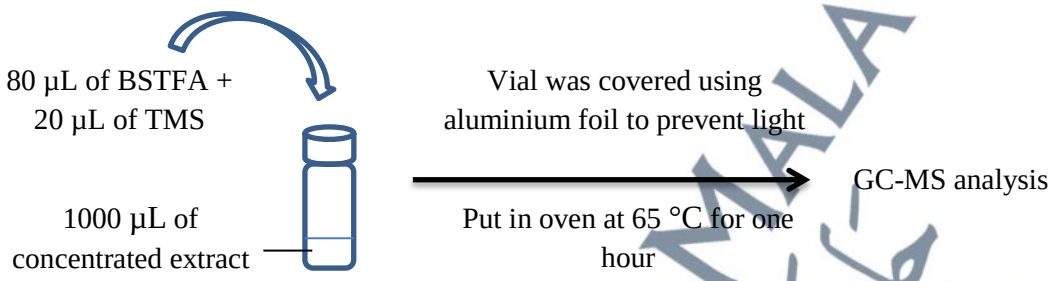


**Figure 3.5:** Flow Chart of Derivatization Step

### 3.2.3.2 Sample Preparation for LC-QToF-MS Analysis

To extract the water-soluble material from Ajwa dates, a total of 50 g of sample was added into a Waring blender containing 100 mL of ultra-pure water and was ground to get a homogeneous sample solution. After blending, the extract was transfered into 250 mL of conical and was left at room temperature for 24 hours. Then the extract was filtered using Whatman No. 1 filter paper into a new 250 mL conical flask to separate the crude sample. An aliquot of filtrate (1.5 mL) was filtered through PTFE membrane filter with 0.2 µm pore size and 13 mm diameter and then injected into LC-QToF-MS. Water sample can be directly injected into liquid chromatography tandem mass and could avoid the use of organic solvents and minimize sample handling, yet increase the sample yield (Borrull et al., 2019). This method was based on Al-Najada & Mohamed (2014) with modifications on solvent used. The sample preparation flow is simplified in Figure 3.6.

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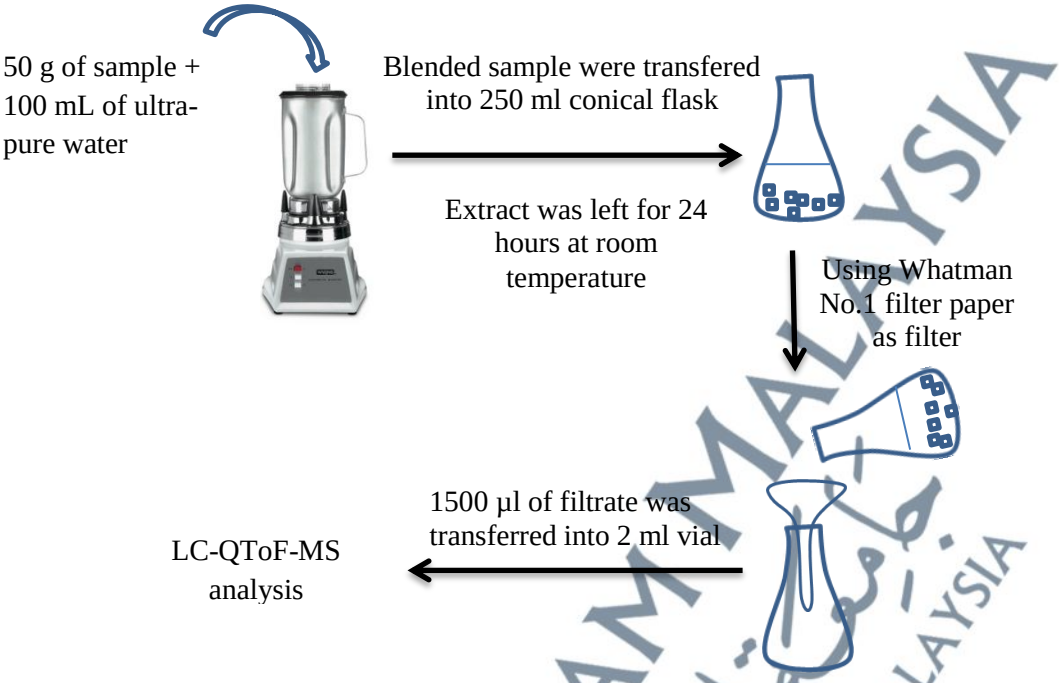


**Figure 3.6**: Sample Preparation of Ajwa Dates Flesh for LC-QToF-MS Analysis

### 3.2.3.3 Sample Preparation for FTIR Analysis

Including Ajwa dates fruit used as main sample in this study, another nine different type and brands of dates fruits were purchased at local market and labeled as shown in Table 3.3. Ajwa dates labeled as K1 was purchased from Egypt Bazaar while Ajwa dates labeled as K4 is the main sample in this study. Safawi and Mariami is a product from Egypt Bazaar and labeled as K2 and K3, respectively. On the other hand, Medjool, which is labeled as K5 was imported from California. Safia, Ameera and Safina are imported from Tunisia and labeled as K6, K7 and K8, respectively. Meanwhile Raziz, labeled as K9 is a product from Saudi Arabia and Sunseed is imported from Iran and labeled as K10. All samples were rinsed with distilled water and squashed prior to analysis. The samples used are shown in Appendix 2.

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**Table 3.3**: Types/Brand of Dates Fruits and the Label.

| Dates Fruits Type/ Brand | Label | Dates Fruits Type/ Brand | Label |
|---|---|---|---|
| Ajwa | K1 | Safia | K6 |
| Safawi | K2 | Safina | K7 |
| Mariami | K3 | Ameera | K8 |
| SAG Ajwa | K4 | Raziz | K9 |
| Medjool | K5 | Sunseed | K10 |

### 3.2.4 Instrumentation

#### 3.2.4.1 GC-MS Analysis

Gas chromatography mass spectrometry (GC-MS) analysis is performed on an Agilent 7890A gas chromatography (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector using a 30 m (length) and 0.25 mm (diameter) column (model DB-5MS UI). The film thickness of the column was 0.25 μm and 5 % phenylmethylpolysiloxane was used as the stationary phase.

The column temperature was set to 80 °C for 3 min, then increased to 250 °C at the rate 7 °C/min and then held for 15 min. The injector temperature was 250 °C; splitless mode was used and the injection volume was 1 μL. The flow rate of the carrier gas was set to 20 mL/min after 2 min. Total run time was 42 min. Mass spectra were obtained from the range m/e 45 to 600 and the electron ionization at 70 eV. The chromatograms of the sample were identified by comparing their mass spectra with NIST/EPA/NIH version 2.0 from Agilent Mass Hunter software. Details are shown in Appendix 3.

### 3.2.4.2 LC-QToF-MS Analysis

Analysis of Ajwa dates fruit was performed on ACQUITY UPLC I-Class system with FTN Sample Manager (WATERS Corporation, MA, USA) instrument. Compounds were chromatographically separated using a column ACQUITY UPLC HSS T3 (100 mm x 2.1 mm x 1.8 μm), maintained at 40 °C. The sample temperature was set to 15°C. A linear binary gradient of water (0.1 % formic acid) and acetonitrile was used as mobile phase A and B respectively. The mobile phase composition was changed during the run as follows: 0 min, 1% B; 0.5 min, 1% B; 16.00 min, 35% B; 18.00 min, 100% B; 20.00 min, 1% B. The flow rate was set to 0.6 mL/min and the injection volume was 10 μL. Gradient setting for mobile phase A and B is referred to Avula et al (2012) with slight modification on the setting and is tabulated in Table 3.4.

The UHPLC system was coupled to a Vion IMS QToF hybrid mass spectrometer, equipped with a lock spray ion source. The ion source was operated in positive and negative electrospray ionization (ESI) mode under the following specific conditions: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120 °C; desolvation gas temperature, 550 °C; desolvation gas flow, 800 L/h, and cone gas flow, 50 L/h. Nitrogen (>99.5%) was employed as desolvation and cone gas. Data were acquired in high-definition $MS^E$ ($HDMS^E$) mode in the range m/z 50 - 1500 at 0.1 s/scan. Thus, two independent scans with different collision energies (CE) were alternatively acquired during the run: a low-energy (LE) scan at a fixed CE of 4 eV, and a high- energy (HE) scan where the CE was ramped from 10 to 40 eV. Argon (99.999%) was used as collision-induced-dissociation (CID) gas. Details are shown in Appendix 4.

**Table 3.4:** Gradient Setting for Mobile Phase A and B

| Time | Flow rate (mL/min) | Solvent A | Solvent B |
|---|---|---|---|
| 0 | 0.6 | 99 | 1 |
| 0.5 | 0.6 | 99 | 1 |
| 16 | 0.6 | 65 | 35 |
| 18 | 0.6 | 0 | 100 |
| 20 | 0.6 | 99 | 1 |

### 3.2.4.3 FTIR Analysis

Analysis on Ajwa dates fruit was carried out using Spectrum 100 Perkin Elmer equipped with a detector of deuterated triglycine sulphate (DTGS), a beam splitter of KBr/Germanium, and connected to the software of the Spectrum operating system (Version 1.1). A thin piece of squashed dates fruits was placed on the attenuated total reflectance (ATR) crystal plate in a controlled temperature (20°C). Then they were scanned for 4 times in the range of 4000–600 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ and were recorded in the form of transmittance. These spectra will be subtracted from background air spectrum. After each scans, a new reference air background spectrum will be taken. The ATR plate will be carefully cleaned with methanol and was dried with a soft tissue before placing in the next sample. Cleanliness could be verified by collecting a background spectrum and will be compared to the previous one. Three readings of each sample were recorded. This method was based on Bureau et al (2009). Details are shown in Appendix 5.

## 3.2.5 Data Analysis

The raw data from GC-MS analysis was exported into the Microsoft Excel as excel spreadsheet (XLS) file. The informations exported into data matrix are the name of compounds (rowset / scores) and the solvents design (columnset / loadings) with

the details of percentage area in the center of data matrix. In this case, the complicated total ion chromatograms (TICs) of chemical compounds identified in Ajwa dates flesh was analysed based on similarity index, retention time and the usage of the compounds (phytochemical activities of the compounds were referred to other studies). This is due to the reason targeting which bioactive compounds in Ajwa dates are associated with human health. The preprocessing method used for this multivariate data is the normalization which to avoid the ambiguity of the peaks intensity in the resolved profiles. This preprocessing method has six options which are area normalization, mean normalization, unit vector normalization, maximum normalization, range normalization and peak normalization. Therefore, mean normalization is preferred on the data set of GC-MS analysis of Ajwa dates and then the Principal Components Analysis (PCA) was performed on the data set using Unscrambler 10.3 (CAMO Software, Norway) to cluster data into the scores and loadings plot.

### 3.3 Metabolites in Urine after Consumption of Ajwa Dates Flesh

#### 3.3.1 Chemicals and Materials

Internal NMR standard; trimethylsilylpropanoic acid (TSP) was purchased from Merck while deuterated water ($D_2O$) was bought from Sigma-Aldrich. Solvents were of GC-MS grade and purchased from Merck (petroleum ether, chloroform, ethyl acetate and methanol) and Sigma-Aldrich (Supelco-33027: N, O-bis(trimethylsylil) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS)).

### 3.3.2 Subjects of Study

Volunteers between 20 to 25 years old with body mass index of 20-25 were selected. In total, 10 healthy volunteers including 5 females and 5 males were enrolled with informed written consent (Appendix 6). This study was approved by the Human Ethics Committee (HEC) of Universiti Sains Islam Malaysia (USIM/REC/2016-14) (Appendix 7). Subjects were required to follow the control diet, which exclude fruits and vegetables, supplements and beverages such as tea, coffee, fruit juice or any bicarbonate drinks during the period of study. Diet diary (Appendix 8) and menu were given to subjects after considering their food allergy as tabulated in Table 3.5.

**Table 3.5:** Diet given to volunteers

| | **Day 1** | **Day 2** | **Day 3** | **Day 4** |
|---|---|---|---|---|
| **Breakfast** | 2 Sausages<br>Bread Roll | 2 Sausages<br>Bread Roll | 2 Sausages<br>Bread Roll | 2 Sausages<br>Bread Roll |
| **Lunch** | Chicken Rice | Chicken Rice | Chicken Rice | Chicken Rice |
| **Dinner** | Chicken Rice | Chicken Rice | Chicken Rice | Chicken Rice |

*Volunteers can take provided drinking water during the study.

### 3.3.3 Collection of Urine Samples

Subjects were required to fast overnight (every day from 10 pm to 8 am). The study had been done for 4 days (Day 1 until Day 4). Urine samples were collected at 7 am and 7 pm on Day 1 (a day before consumption of Ajwa dates flesh). On Day 2, urine samples were collected at 7 am as blank (0 hours) before all subjects were given 7 Ajwa dates to be consumed. Subsequently, urine were collected at 4, 8, 12 and 24 hours after the consumption of Ajwa dates, which were at 11 am, 3 pm, 7 pm on Day 2 and at 7 am on Day 3, respectively. The urine collection continued at 7 pm on Day 3, and twice time (7 am and 7 pm) on Day 4. The collected samples were kept in cold

box during collection and transportation before kept immediately in a freezer at -20°C in the laboratory. According to Laparre et al (2017), metabolites are mostly preserved if the samples are frozen immediately at or below temperature of -20 °C. Summary of urine collection is illustrated in Table 3.6.

**Table 3.6**: Collection of Urine Sample

| | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|
| 7 am | ✓ | ✓ (0h) | ✓ (24h) | ✓ |
| 11 am | | ✓ (4h) | | |
| 3 pm | | ✓ (8h) | | |
| 7pm | ✓ | ✓ (12h) | ✓ | ✓ |

### 3.3.4 Sample Preparation for Urine Sample

#### 3.3.4.1 $^{1}$H-NMR Analysis

Samples preparation and $^{1}$H-NMR analysis were performed as described by Walsh et al. (2007) with some modifications on the sample preparation where in this study we prefer less preparation without buffer. Briefly, the urine samples were thawed at room temperature (25°C) before the analysis. An aliquot of urine (1000 µl) was centrifuged at 5 000 rpm for 5 min to remove the precipitate. The supernatant (500 µl) was mixed with internal standard TSP (10 µl; 5 mg of TSP dissolved in 1000 µl of $D_2O$) and $D_2O$ (250 µl). Prepared sample was then well mixed and the samples (600 µl) were transferred into a 5 mm $^{1}$H-NMR sample tube (Norell). $^{1}$H-NMR analysis was performed at Malaysia Genome Institute (MGI). Figure 3.7 illustrates the sample preparation of urine.

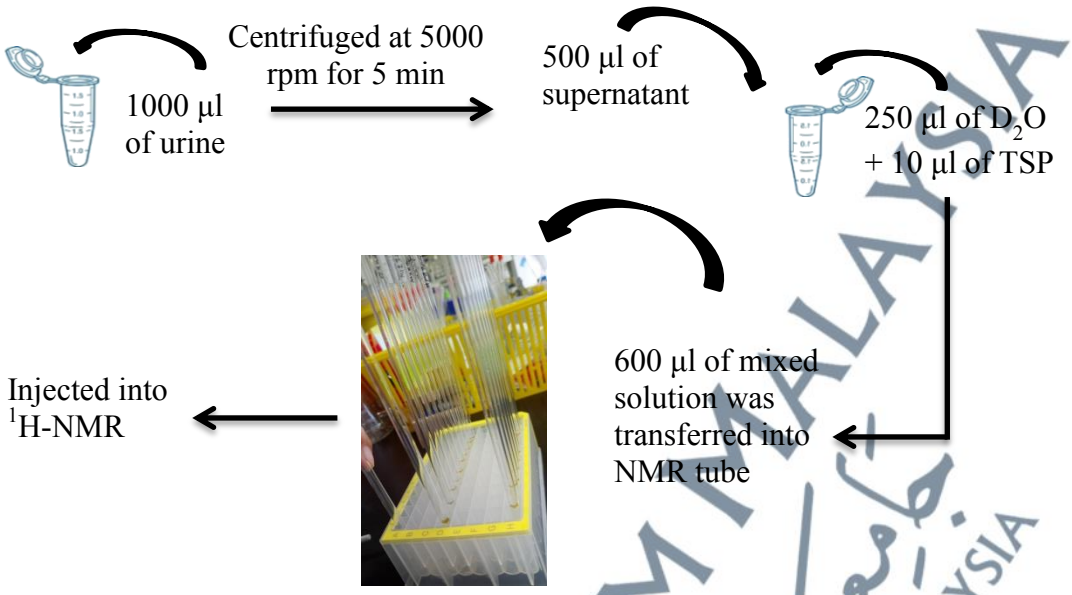


**Figure 3.7:** Sample Preparation of Urine for $^1$H-NMR Analysis

### 3.3.4.2 GC-MS Analysis

#### 3.3.4.2.1 Sample Preparation

Urine samples from one volunteer out of ten was selected to be analysed using GC-MS. The volunteer was carefully chosen based on $^1$H-NMR spectrum, which gave the best profiles in term of clear variation of peak intensity from 0 hour to 24 hours. Sample preparation is shown in Figure 3.8. The sample was thawed at room temperature (25 °C) prior to analysis. Acid-base extraction was chosen for urine analysis using GC-MS. The pH strip was dipped into the urine samples before the extraction took place to make sure the urine samples are neutral (pH 7). A 30 ml of urine sample was filtered using No 1 Whatman filter paper into a 100 ml separating funnel. Then 50 ml of petroleum ether (PET) was added into the same separating funnel. The mixture was shaken vigorously to allow the metabolites in urine sample migrate into PET solvent. Both urine sample and PET layers were collected separately

in a 100 ml beaker. A few drops of 1M hydrochloric acid (HCL) were added into the beaker that contain urine sample to acidify the urine to pH 3.

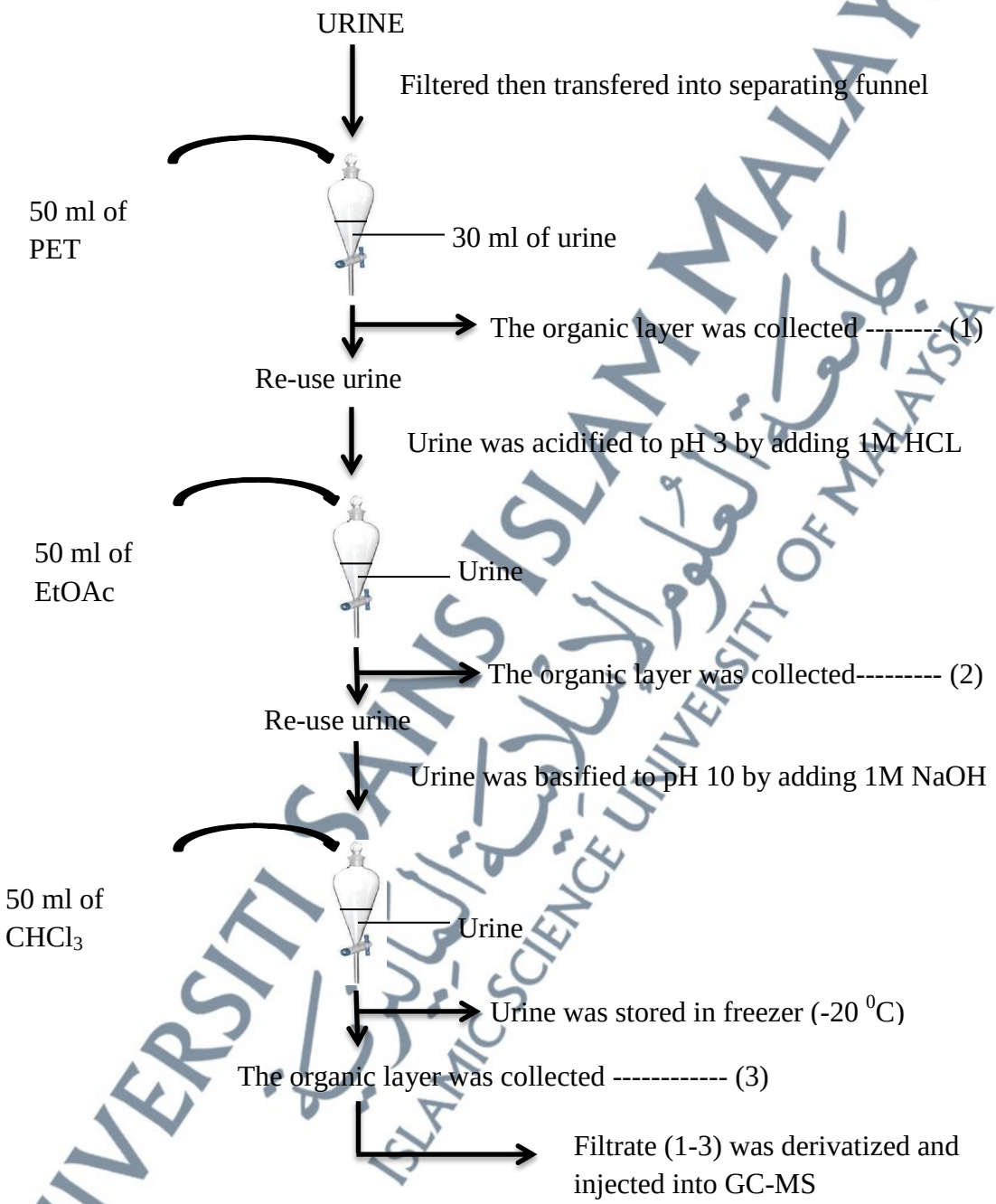


**Figure 3.8:** Flow Chart of Urine Sample Preparation for GC-MS Analysis

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The acidified urine was transferred into a clean 100 ml separating funnel and 50 ml of ethyl acetate (EtOAc) was added into the same separating funnel. The mixture was shaken vigorously to allow the metabolites in urine sample migrate to EtOAc. Both urine sample and EtOAc layers were collected separately in a 100 ml beaker. A few drops of 1M sodium hydroxide (NaOH) were added into the urine sample to basify the urine sample to pH 10. The basified urine sample was mixed with 50 ml chloroform ($CHCl_3$) in a clean 100 ml separating funnel. After vigorous shaking, both urine sample and $CHCl_3$ layers were collected separately in a 100 ml beaker. All three organic layers (PET, EtOAc and $CHCl_3$) were then vaporized to concentrate the samples. Concentrated samples were derivatised before injected into GC-MS.

#### 3.3.4.2.2 Derivatization Process

The derivatization step for urine sample was followed as the same derivatization step in the subtopic 3.2.3.1.3. The samples were then analysed using GC-MS.

### 3.3.4.3 LC-ToF-MS Analysis

The same urine sample used in GC-MS analysis was selected for LC-QToF-MS analysis. This method followed approach carried out by Martinez-Lopez et al (2014). The sample used in Martinez-Lopez et al (2014) is cocoa product while in this study the flesh of Ajwa dates is directly consumed. Briefly, the urine samples were thawed at room temperature (25°C ) before the analysis and vortexed using vortex mixer 3000 (Labmart, Malaysia) to homogenize the sample. An aliquot of urine (2.0 mL) was filtered using PTFE membrane filter with 0.2 μm pore size and 13 mm diameter and then centrifuged at 13 000 rpm for 5 min to remove the precipitate. The supernatant

(1.0 mL) was transferred into a vial (2.0 mL) and diluted with an organic solvent; methanol (0.5 mL) and then well mixed. The prepared sample was directly injected to LC-QToF-MS. Figure 3.9 shows the flow of urine sample preparation.

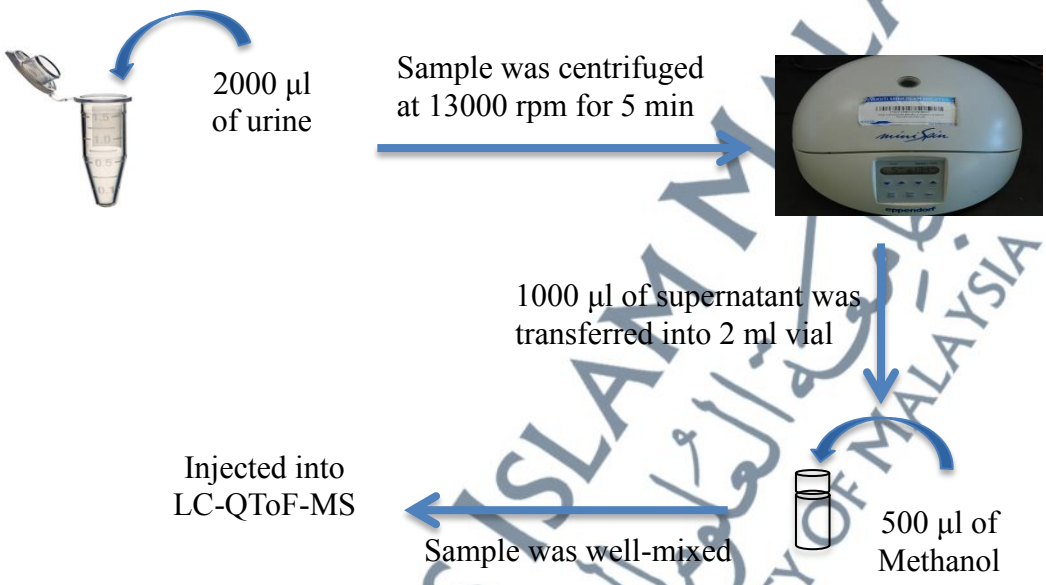


**Figure 3.9:** Flow Chart of the Urine Sample Preparation

### 3.3.5 Instrumentation

#### 3.3.5.1 $^{1}$H-NMR Analysis

NMR analysis of urine samples was carried out on a Bruker Ascend spectrometer (BRUKER Corporation, United State) operating at 700 MHz and equipped with a 5mm TCI cryogenically cooled probe (Appendix 9) a triple resonance NMR inverse probe optimized for $^{1}$H/$^{13}$C/$^{15}$N experiments. In addition, this instrument is also fitted with a SampleCase autosampler which includes a random-access, 24 sample automation system. The acquisition temperature (TE) was set to 298 K. All spectra were obtained with 32 scans (NS) after application of 4 dummy scans (DS). The relaxation delay (D1) was set to 2 s with the acquisition time (AQ) of 1.46 s.

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Thirty-two Free Induction Decays (FIDs) (TD) were required into 32768 (32 K) complex data points using a spectral width (SWH) of 11194.03 Hz. An exponential line-broadening function of 0.3 Hz was applied to the FID prior to Fourier Transformation. All spectra were processed manually phased, calibrated and baseline corrected within TOPSPIN 1.3 software. Chemical shift was internally referenced to TSP (δ 0.0 ppm).

### 3.3.5.2 GC-MS Analysis

GC-MS analysis of the sample was carried out using Agilent Intuvo 9000 GC system with Agilent 5977B and 7693 autosampler are equipped with Intuvo HP5-MS-UI column (30m x 0.25 mm i.d. x 0.25 μm film thickness; Agilent), nonpolar column with very low bleed characteristics. Helium was used as the carrier gas with flow rate of 1 ml/ min. Initial oven temperature was set at 80°C for 1 min, then the temperature was ramped at a rate of 15°C $min^{-1}$ to 200°C and hold for 1 min, and finally the temperature was increased to 270°C at 10°C $min^{-1}$ and being hold at that temperature for 10 min. Splitless injection was used and the solvent delay was set up for 4 min and omitted from the final chromatogram.

### 3.3.5.3 LC-QTOF-MS Analysis

Urine analysis was performed on ACQUITY UPLC I-Class system (WATERS Corporation, MA, USA) instrument. The same setting of LC-QToF-MS for Ajwa dates flesh sample (subtopic 3.2.4.2) was used for urine analysis.

### 3.3.6 Data Analysis

#### 3.3.6.1 ChenomX and Human Metabolome Database (HMDB)

Metabolites identification was accomplished by using $^{1}$H-NMR while Chenomx NMR suite 8.2 (Chenomx Inc., Edmonton, Canada) was used for spectral binning (size of bin = 0.04 ppm) in multivariate data analysis. Prior to integration, the water region was removed. Chemical shifts in $^{1}$H-NMR spectra were compared with reference spectra in the Human Metabolome Database (HMDB) (Wishart et al., 2013).

#### 3.3.6.2 Chemometrics

The raw data from $^{1}$H-NMR, GC-MS, and LC-QToF-MS analysis was exported into the Microsoft Excel as excel spreadsheet (XLS) file. The informations exported into data matrix for GC-MS and LC-QToF-MS analysis are the name of compounds (rowset / scores) and the solvents design (columnset / loadings) with the details of percentage area in the center of data matrix. Meanwhile for $^{1}$H-NMR analysis, the chemical shift (after binning) is exported to columset / loadings while urine time collection is exported to rowset / scores into the data matrix. The details in the center of this data matrix are the intensity of the metabolites peaks. In this case, the complicated total ion chromatograms (TICs) of chemical compounds identified in Ajwa dates flesh using GC-MS and LC-QToF-MS analysis were analysed based on similarity index, retention time and the usage of the compounds (phytochemical activities of the compounds were referred to other studies). This is due to the reason targeting which bioactive compounds in Ajwa dates are associated with human health. The preprocessing method used for all multivariate data is the normalization which to avoid the ambiguity of the peaks intensity in the resolved profiles. This preprocessing method has six options which are area normalization, mean normalization, unit vector

normalization, maximum normalization, range normalization and peak normalization. Therefore, mean normalization is preferred on the data set of GC-MS and LC-QToF-MS analysis of Ajwa dates while maximum normalization is preferred on the data set of $^{1}$H-NMR analysis. Then the Principal Components Analysis (PCA) was performed on all data set using Unscrambler 10.3 (CAMO Software, Norway) and the results are presented in score and loading plots.

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