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Quality evaluation of the physical properties, phytochemicals, biological activities and proximate analysis of nine Saudi date palm fruit varieties

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ABSTRACT

The date palm fruit (*Phoenix dactylifera*) is one of the earliest crops to be cultivated in the Middle East. Many varieties of dates are available and being marketed at various price ranges. However, there is no standardized approach available to verify the quality of Saudi date palm fruits. The current study aimed to assess the variations in the quality of different varieties of Saudi dates on the basis of the metabolite composition, proximate analysis, physical characteristics and biological activities. Nine date palm varieties Berni, Halaoua, Shalabi, Sogaai, Sukkari, Nebtat Ali, Anbara, Ajwa and Medjoul were evaluated via the Proton Nuclear Magnetic Resonance (^{1}H NMR) based metabolomics approach. The DPPH and nitric oxide (NO) scavenging abilities, NO inhibition via the cell-based approach and total phenolic content (TPC) were determined. The physical features including dimension, seed/weight ratios and color variation also were evaluated. The proximate composition was carried out using the protocol ascribed by AOAC method. Metabolomics approach was successfully being applied in discriminating date palm varieties. Un-targeted metabolite profiling was achieved based on the acquired NMR data. Different trend of biological activities were displayed. The Ajwa dates which was the smallest, shortest in length and darkest colored Saudi date palm fruit demonstrated the highest percentage of NO inhibition/scavenging. Based on the variation in physical features, biological activities, metabolites content and proximate analysis, no noticeable correlation were observed between the market price and Saudi date palm variety qualities.

1. Introduction

Date palms with the scientific name of *Phoenix dactylifera* L. belong to the Arecaceae family. The date palm fruit (DPF) is one of the earliest crops to be cultivated in the Middle East. DPF are ovoid in shape with a soft and sugary pericarp. Saudi Arabia is one of the prominent countries that exports dates and has approximately 7 to 8 million palm trees (Al-Hooti et al., 1997). Nearly 400 different varieties of dates are available from Saudi Arabia, yet, only a limited number of date varieties have been utilized for scientific purposes. According to Baliga et al. (2011), the shape of DPF and their organoleptic properties can indicate the different varieties. In addition, the color of the DPF skin is also key aspect that affects consumer preference in the selection of DPF (Al-Jasass et al., 2015).

The quality of dates is typically determined on the basis of their physical properties, such as color, shape, size and texture, whereas the nutritional content is assessed with reference to their chemical properties, and the sensory attributes including the flavor of the DPF. Earlier studies have reported that the color of the DPF varies significantly according to the maturity stage and varieties of dates (Amoro's et al., 2015; Noui et al., 2014). Likewise, physical evaluations are frequently used to provide the criteria for the preliminary evaluation for suppliers in sorting and grading the DPF. Furthermore, Said et al. (2014) showed that the physical features

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including flesh weight, seed weight, the length and diameter of dates were significantly different from one variety to another.

Conventionally, DPFs have been used to treat various illnesses, for instance to treat sore throat and also to relieve fever (Chao and Krueger, 2017). The health benefits of DPF were validated based on experimental data that have demonstrated inhibition or suppression of the occurrence of many diseases. From the medicinal viewpoint, DPF have been shown to retain several biological activities, including antioxidant, anti-inflammatory and anti-carcinogenic activities (Al-Mamary et al., 2014; Farag et al., 2014; Saleh et al., 2011). Previously, several studies have established that the biological activities of DPF vary with regard to the variety (Farag et al., 2014; Saleh et al., 2011). This has triggered an interest in evaluating the variations of the DPPH and NO scavenging abilities, NO inhibition via the cell-based approach and total phenolic content (TPC) among these selected Saudi dates.

Currently, in fulfilling the demands and preferences of consumers in selecting food and food products that possess health promoting effects, knowledge regarding the type and concentration of health protective compounds available in food is crucial. Proximate analysis is normally applied in the food industry, particularly with respect to food labeling to describe the nutritional information (Cunningham and Sobolewski, 2011). Similarly, total soluble solid (TSS) analysis can be performed on the juices of fruits. This analysis indicates the amount of total soluble sugars, including sucrose, fructose and glucose together with other soluble components namely, organic minerals and soluble pectin. This information is valuable for the food industry to verify the quality of food, which ultimately influence the appreciation of certain foods in the market.

Currently, there is no established scientific method available for pricing and grading DPF except an examination of the external physical characteristics. Moreover, there has been a lack of scientific data regarding the physiochemical, nutritional quality and also phytochemical composition of DPF and the association of these characteristics with the marketed price. Therefore, this study was carried out by focusing on nine selected varieties of Saudi Arabian DPF ; Berni (BR), Halaoua (HL), Shalabi, (SB), Sogaai (SG), Sukkari (SK), Nebtat Ali (NB), Anbara (AN), Ajwa (AJ) and Medjoul (MJ). These varieties are listed based on their marketed prices; from the least to most expensive. The details on the prices are available in Table S1 in the Supplementary. The selected varieties were chosen in accordance to their high commercialization values and consumers' preferences. We also used a metabolomics approach to identify the factors that contribute to the variations in the quality of various varieties of dates and to determine whether the quality corresponded with the marketed price. The quality was evaluated on the basis of the metabolite composition, proximate analysis, physical characteristics and biological activities.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jssas.2018.08.004.

2. Materials and methods

2.1. Chemicals and reagents

Deuterated methanol-d_4 (CD_3OD, D 99.8%), non-deuterated KH_2PO_4, sodium deuterium oxide (NaOD), trimethylsilylpropionic acid-d4 sodium salt (TSP), deuterium oxide (D_2O, D 99.9%), concentrated sulphuric acids (H_2SO_4), mix catalyst (96% sodium sulphate anhydrous + 3.5% cuprum sulphate + 0.5% selenium dioxide), 45% and 0.313 N sodium hydroxide (NaOH), distilled water, indicator solution (0.2% methyl red + 0.1% methylene blue in 96% ethanol), 2% boric acid, 0.05 N and 0.25 N sulphuric acid, solvent petroleum ether, alcohol, and also Folin-Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Curcumin, sodium carbonate, quercetin, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitroprusside (SNP), lipopolysaccharide (LPS), phosphate buffered saline (PBS) and recombinant murine IFN-γ were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The cell culture media, Dulbecco's Modified Eagle's Medium (DMEM) containing both HEPES and L-glutamine with phenol red, and that without phenol red, penicillin-streptomycin antibiotic solutions, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo lium bromide (MTT) and triple Express enzyme were obtained from Gibco/BRL Life Technologies Inc. (Eggenstein, Germany).

2.2. Fruit materials

The nine selected varieties of Saudi Arabian DPF; Berni (BR), Halaoua (HL), Shalabi, (SB), Sogaai (SG), Sukkari (SK), Nebtat Ali (NB), Anbara (AN), Ajwa (AJ) and Medjoul (MJ) has been attained from the same farm in Madinah al-Munawarrah located at the North-Western part of Saudi Arabia. The landmark Global Positioning System (GPS) coordinates of the farm are 24̊29′51″N 39̊36′10.4″E. All DPF were attained at tamr stage and identical treatment was given. Six biological replicates of three DPF were analyzed for each variety (for freedom of defects or physical injury) from the dates collected.

2.3. Physical characterization of DPF

For the color analysis, it was determined using a Hunter Lab Ultra Scan PRO colorimeter attached with Easy Match QC software. The results were expressed following the CIELAB system which using the rates of *L, a* and *b* parameter. *L* values measured lightness with a range from 0 for black until 100 for white, respectively. The *a* values ranging from −60 for greenness to +60 for redness and *b* values; from −60 for blueness until +60 for the degree of yellowness.

The dimensions of the DPF which consisted of length and width were measured using Vernier Caliper with a precision of 0.01 mm meanwhile, the weight of DPF and the seed was acquired using an analytical balance with a precision of ±0.0001 mg (Cunningham and Sobolewski, 2011).

2.4. Sample preparation

The freeze dried DPF, which comprised of both the flesh and peel (6.0 g) were extracted with 60 mL of absolute ethanol through ultrasound assisted extraction method using a Nexul Ultrasonic Cleaner (NXP1002), for 1 h without heating (Khoo et al., 2015). Following this, the extraction process was continued by soaking in the solvent at room temperature for 24 h (Abdul-Hamid et al., 2015). The extracts were then being filtered using Whatman filter paper No. 1 in a Buchner funnel, and were concentrated to dryness under vacuum with a rotary evaporator. All samples were then stored in the dark at 4 ℃ until further analysis.

2.5. Nitric oxide (NO) inhibitory activity and cell viability

Briefly, RAW 264.7 murine macrophages cells were challenged with the triggering agents of recombinant murine IFN-γ and 10 μg/ml lipopolysaccharide. The stable nitric oxide conversion product, nitrite (NO_2^-) was measured using the freshly prepared Griess reagent (1% sulfanilamide, 0.1% N-(l-naphthyl)-ethylene diamine dihydrochloride, 2.5% H_3PO_4) at room temperature (Abas et al., 2006). Curcumin served as positive control. The absorbance was taken at 550 nm using SpectraMax Plus UV–Vis microplate reader (Molecular Devices, GA, USA).

After the removal of media, 100 μL of complete DMEM was pipetted into the wells. Then, 20 μL of MTT solution were added. The cells were incubated at 37 °C in 5% CO_2 for 4 h. The DMSO was added to dissolve the formazan salt. The absorbance was read at 570 nm (Abas et al., 2006).

2.6. NO scavenging activity

The 60 μL of 10 mM SNP in phosphate buffered saline was mixed with 60 μL of the DPF extracts in the 96 well plates and incubated for 150 min. The extract was mixed with an equal volume of freshly prepared Griess reagent. The absorbance was then being measured at 550 nm. Gallic acid acted as the positive control (Tsai et al., 2007).

2.7. DPPH scavenging assay

The assay was conducted using 96 well plates added with 50 μL of sample extracts followed with the reaction with 100 μL of DPPH. The plates were incubated in dark for 30 min. The absorbance was then taken at 515 nm (Khoo et al., 2015). Quercetin served as the positive control.

2.8. Total phenolic content (TPC) assay

The TPC of date extracts was assessed using Folin-Ciocalteu method utilizing gallic acid as reference compound also based on the protocols reported by Khoo et al. (2015).

2.9. NMR measurement

Briefly, the sample preparation for NMR measurement was performed following the method reported by Maulidiani et al. (2015). Ten milligrams of the freeze dried, ethanolic DPF extracts were transferred into a 1.5 mL Eppendorf tubes containing 375 μL of CD_3OD and 375 μL of KH_2PO_4 buffer in D_2O (pH 6.0) containing 0.1% TSP. The pH was adjusted using NaOD solution. The tubes were then vortexed and ultra-sonicated for 10 min, followed with centrifugation at 13,000 rpm for 10 min. The ^{1}H NMR analysis was performed at 26̊C utilizing a Varian INOVA 500 MHz NMR spectrometer (Varian Inc., California, USA). Gradient shimming was accomplished preceding the NMR data acquisition and the D_2O was utilized as an internal lock. Suppression of the large water resonance was achieved by applying a PRESAT pulse sequence to the ^{1}H NMR data acquisition; using low power selective radiation at 4.85 ppm throughout the recycle delay. For each sample, the required time was 4.29 min recording 64 scans with an acquisition time, pulse width (90̊ pulse angle), relaxation delay and spectral width of 2.045 s, 8.6 ms, 2.0 s, and 16 ppm respectively. The chemical shifts were referenced to the internal standard, TSP = 0.0 ppm. The metabolite identification was further supported by data obtained from 2D J-resolved (JRES) NMR experiment. The JRES spectra was achieved at 8012.8 Hz spectral width in both dimension with 256 t1 increments. The resulting JRES spectra was tilted at 45̊ viewing the chemical shift and coupling constant axes in orthogonal means; and was then symmetrized along F1 directions (Gogna et al., 2015).

2.10. Multivariate data analysis

The acquired free induction decays (FID) from ^{1}H NMR were phased, base-line corrected and binned using Chenomx software (v. 5.1, Alberta, Canada). Chenomx NMR suite was also utilized for characterization of metabolites. A line broadening of 1.0 Hz was applied. The regions containing water (4.70 to 4.90 ppm) and residual methanol (3.28 to 3.32 ppm) were excluded from all the spectra. The binned ^{1}H NMR data were then subjected to MVDA performed with SIMCA-P+ version 13.0 (Umetrics AB, Umeå, Sweden) software. Additional analysis was carried out using MetaboAnalyst data approach (Xia et al., 2009). The peak picking algorithm from MestReNova software (v. 6.0.2, Mestrelab Research, Santiago, Spain) was also applied for NMR data assessment.

2.11. Total soluble solid (TSS) and proximate composition analysis

The Total soluble solid was verified using digital Refractometer ATAGO (Atago, Tokyo, Japan). The results were expressed as degree Brix (°Brix) (Jamil et al., 2010).

The determination of moisture, protein, fibre, ash and fat contents of the DPF samples were performed following the official methods ascribed by Association of Official Analytical Chemistry (AOAC 2005) (Jamil et al., 2010).

2.12. Statistical analysis

Results are reported as the mean of six 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. Physical characteristic of dates

The physical characteristics of the DPF were assessed in the current study included the dimensions of the DPF (length and diameter), color with *L, a* and *b* values and the seed/weight mass ratio. These data are tabulated in Table 1. There were significant differences among the dates with respect to the dimension parameter especially for the AJ variety. The AJ, which was among the most expensive varieties, had the smallest and the shortest date size among the varieties examined. The diameter and length of this variety were 14.6 mm and 26.4 mm, respectively. The AN dates were found to have the longest length, 45.1 mm, among the nine tested samples.

With regard to the seed per fruit mass ratio, there were no significant differences among HL, NB and SK. Furthermore, the SG and AN displayed the highest seed/weight mass ratio with no significant difference at 16.8 g and 16.5 g, respectively. The AJ variety displayed the lowest seed per fruit mass ratio of 6.1 g.

The *L* value for AJ was determined to be 26.40 which was significantly different from that of AN. Having the lowest *L* value indicated that AJ was darker in appearance than the other studied dates. Conversely, the *L* value for the NB variety of date was the highest, 37.3 and was significantly different from those for the rest of the DPF varieties. This indicated that this variety was pale and brighter in color than the others. In addition, the value of *a* also varied among these varieties of dates. The lowest value was observed for AJ (1.1) whereas MJ showed the highest value (9.9), and the values for the other varieties were within the range of 3.4 to 8.3. With respect to *b* parameter, the AJ again displayed the lowest value (0.4) and the highest b value, 11.7 was observed for NB. There were only slight differences among the other varieties. The positive values for *a* and *b* indicated the absence of green and blue tints in the DPF. The results obtained were consistent with the data previously reported by Al-Jasass et al. (2015), which examined the colors of Moroccan DPF. According to Biglari et al. (2008), the difference in colors of the dates is mainly due to genetic variations.

Table 1
The phenotypic data analysis of Saudi date palm fruit varieties focusing on color, dimension and seed/weight mass ratios.

Different varieties of date palm fruits						
Samples	Dimension			Color		Seed/weight mass ratio
	Length (mm)	Diameter (mm)	*L* (lightness)	*a* (redness)	*b* (yellowness)	
SK	26.7 ± 1.4^{c}	20.4 ± 1.5^{a}	29.3 ± 3.0^{a}	7.5 ± 3.0^{a}	6.2 ± 1.2^{bc}	11.5 ± 3.4^{a}
HL	38.9 ± 3.2^{a}	18.9 ± 1.6^{ab}	26.3 ± 1.1^{a}	4.2 ± 2.6^{a}	3.1 ± 1.5^{bc}	12.3 ± 2.9^{a}
AJ	26.4 ± 1.7^{c}	14.6 ± 2.6^{c}	20.9 ± 2.1^{b}	1.1 ± 2.1^{b}	0.4 ± 0.2^{d}	6.1 ± 0.2^{b}
NB	31.7 ± 3.5^{b}	20.0 ± 1.6^{ab}	37.3 ± 3.5^{d}	8.1 ± 0.9^{a}	11.7 ± 2.2^{a}	11.6 ± 5.0^{a}
MJ	35.6 ± 2.1^{ab}	17.3 ± 3.0^{b}	31.6 ± 2.9^{c}	9.9 ± 2.0^{ac}	6.2 ± 1.8^{bc}	10.1 ± 2.4^{ab}
SB	35.3 ± 4.2^{ab}	16.8 ± 3.8^{b}	28.2 ± 3.1^{a}	5.1 ± 3.1^{a}	4.1 ± 2.1^{bc}	7.5 ± 1.8^{ab}
SG	38.1 ± 3.3^{a}	20.6 ± 1.3^{a}	30.5 ± 4.1^{c}	6.5 ± 4.1^{a}	8.5 ± 2.1^{ab}	16.9 ± 0.2^{c}
AN	45.1 ± 1.9^{d}	19.8 ± 3.3^{d}	23.4 ± 1.7^{b}	3.4 ± 1.7^{ab}	0.6 ± 0.7^{d}	16.5 ± 0.8^{c}
BR	37.7 ± 2.1^{a}	16.5 ± 3.3^{bc}	31.3 ± 3.5^{c}	8.3 ± 1.1^{a}	5.1 ± 2.1^{bc}	7.4 ± 0.6^{ab}

[a] Value = mean ± SD. Value in the same column with different superscript letter differ significantly at 5% probability level.

3.2. Spectral features and assignment of metabolites

In the current study, a total of 51 metabolites were tentatively characterized by nuclear magnetic resonance (NMR) spectrometry. Visual inspection of the ^{1}H NMR spectra of the extracts showed the presence of amino acids, sugars and organic acids, with the most intense signals being in the sugar regions. The signals belongs to the amino acids were characterized as alanine, arginine, asparagine, proline, phenylalanine, glycine, cysteine, serine, isoleucine, leucine and glutamine (Fig. S1A in Supplementary). This was in agreement with the findings reported by the earlier studies (Diboun et al., 2016; Farag et al., 2014, 2016). The high field signal at 0.88 ppm was assigned to fatty acids. The presence of fatty acids in DPF was consistent with the results reported by Aris et al. (2013) and Bouhlali et al. (2015). Furthermore, several metabolites also have been characterized in the aromatic regions as shown in Table S2 in Supplementary based on the reported data by Georgiev et al. (2011), Gogna et al. (2015), Khoo et al. (2015), Maulidiani et al. (2015), Mediani et al. (2017), and Pramai et al. (2018). The spectral data for the identified metabolites were obtained based on the comparison with the previous literature data and the search from Chenomx database. Furthermore, the assignments of the compounds were further supported by the results of 2D J-resolved NMR spectra (Fig. S1B in Supplementary). The identified metabolites with respective ID are presented in Table S2 in the Supplementary.

3.3. Differentiation of the varieties of Saudi DPF

In an attempt to visualize the subtle pattern of similarities and variations among the DPF samples with respect to the market price, Principal Component Analysis (PCA) was utilized (Farag et al., 2014). The multivariate data analysis (MVDA) performed on the binned integrals of the ^{1}H NMR data revealed a significant separation among the samples. The PCA score plot comprising six biological replicates of nine varieties of Saudi dates was acquired as shown in Fig. 1A. However, to obtain a clearer presentation of the clustering, the average binned data for six biological replicates were calculated and PCA was performed as shown in Fig. S2 Supplementary. Six varieties of DPF were separated from NB, BR and SK by PC1. The discrimination of the varieties was consistent with the previously reported data of metabolomics study performed on DPF samples (Diboun et al., 2016; Farag et al., 2014). The first two components, PC1 and PC2 explained 77.9% and 11.4% of the total variance, respectively. Nonetheless, the PCA score plot was not able to discriminate the DPF according to the market price, which suggested that the varieties predominated over the price range. The PCA loading plot describes the discriminatory metabolites that resulted in the segregation as shown in Fig. 2B. This plot revealed that lysine, serine, glycine, GABA and sucrose accounted for the clustering of SK (PC1 lower right quadrant) from the other varieties. Another striking observation was that the most expensive variety, AJ, and the cheapest one, HL, was tightly clustered in the PC1 upper left quadrant. Among the metabolites that were responsible for this were catechin, cysteine, maleic acid, succinic acid and glutamine. With respect to the market price, distinct clustering can be observed for MJ and AN, the expensive varieties, and also for HL and BR, the cheapest of the studied DPF. It is therefore plausible to suggest that the variation in the phytochemical composition in Saudi DPF was not correlated with the marketed price.

3.4. DPPH inhibitory activity of DPF extracts

Based on the results in Table 2, the MJ dates showed a significantly higher DPPH radical scavenging activity, 40.0% inhibition, compared to other varieties. No significant differences were observed among the AN, SG, SB and BR dates, which demonstrated DPPH percentage inhibition values of 31.5, 30.2, 30.7 and 29.6, respectively. SK demonstrated the least DPPH inhibition at 22.7%. Quercetin, which served as positive control, demonstrated DPPH inhibition of 96.5% at 125 μg/mL. Several scientific studies have demonstrated that DPF have substantial antioxidant activity (Bouhlali et al., 2015; Farag et al., 2014; Mohamed et al., 2016). Earlier studies by Mohamed et al. (2016) revealed that the variety plays a role in the potency of the DPPH scavenging activity shown by DPF. However, the results obtained in the current study demonstrated the relatively weak DPPH scavenging activity of DPF. The results were in agreement with those reported by Al-Harrasi et al. (2014) which highlighted the antioxidant activity shown by the different varieties of Omani DPF. In this study, some of the samples demonstrated inhibition of less than 50%. The possible explanation for the observed differences in the DPPH inhibition behavior may be due to differences in the extraction method, agricultural practices and storage practices as suggested by Lee et al. (2014). Furthermore, Allaith et al. (2008) showed a remarkable reduction in the antioxidant activity of Bahraini DPF as a function of the stage of DPF maturity. Higher radical scavenging activity was displayed by unripe DPF compared to the tamar (ripe) stage. This may be one of the explanations for the low DPPH inhibition shown in the current study. DPF demonstrate a comparatively high amount of tannins during the greenish and towards the completion of the color maturation stages until just at the beginning of the ripening (Al-Hooti et al., 1997; Saleh et al., 2011). It is likely that the decrease in the antioxidant activity can be mainly attributed to the decrease in tannin contents.

3.5. Total phenolic content (TPC)

The determined total phenolic content (TPC) varied from 3.23 to 3.35 mg GAE/100 g dried sample weight (Table 2). This range of

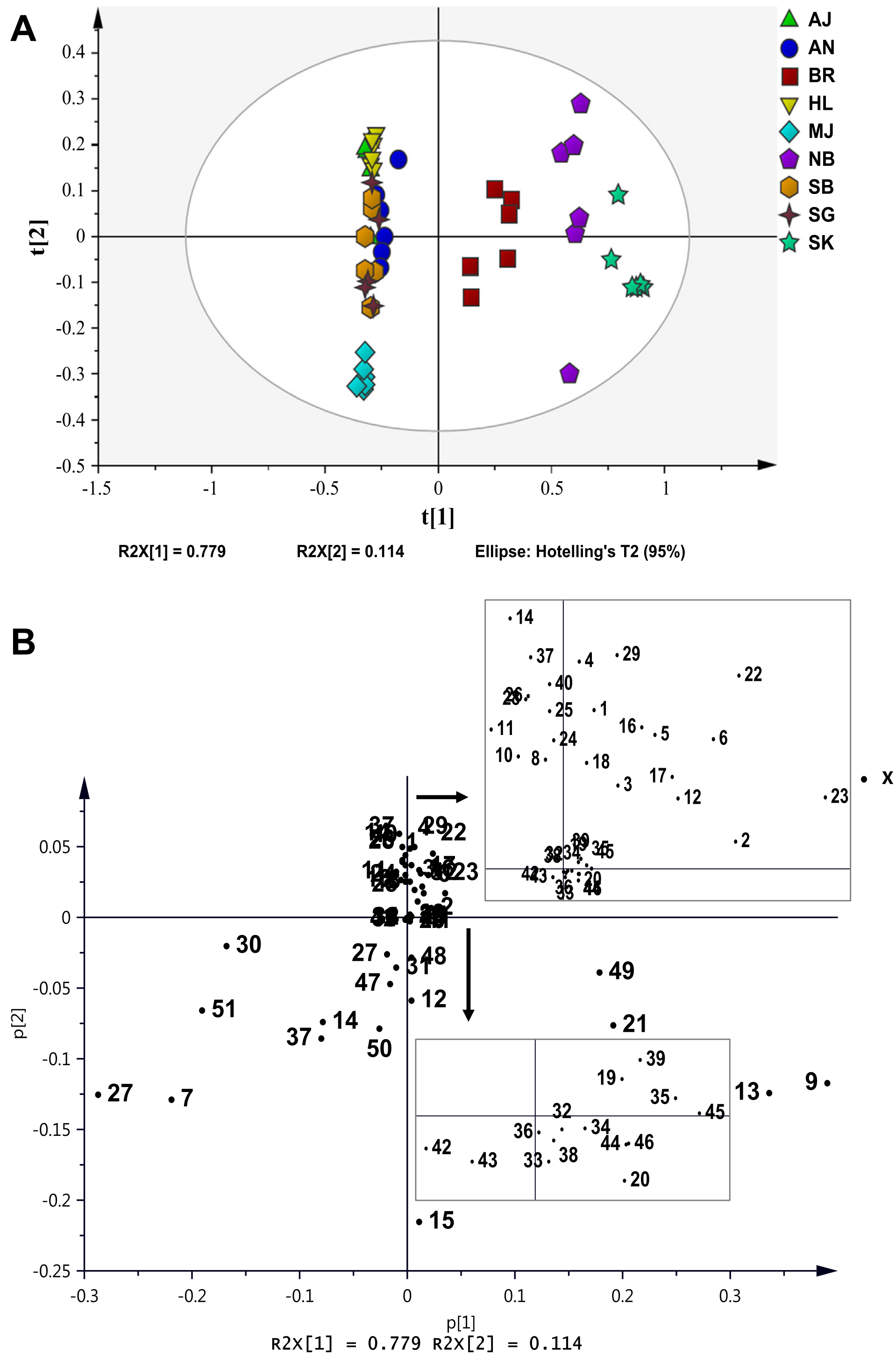

Fig. 1. The principal component analysis (PCA) of nine different varieties of Saudi dates analyzed using ^{1}H NMR data (n = 6). The selected date varieties were Berni (BR), Halaoua (HL), Shalabi, (SB), Sogaai (SG), Sukkari (SK), Nebtat Ali (NB), Anbara (AN), Ajwa (AJ) and Medjoul (MJ) (A) score plot of PC1 and PC2 scores (B) corresponding loading plot representing influential metabolites.

results was consistent with those revealed by Mansouri et al. (2005), who reported that the range of total phenolic content in the dates was between 2.49 and 8.36 mg GAE g sample. The result showed that HL contained the highest TPC. This value was followed by those for BR, SG, NB and SK in a decreasing manner. However, AJ had the lowest TPC, which was significantly different ($p < 0.05$) form the other five date varieties. The results obtained here contrasted with those reported in Saleh et al. (2011) and Mohamed et al. (2016), which showed higher amounts of TPC in the AJ and SK varieties. Our results were, in agreement with those reported by Al Farsi et al. (2005).

The TPC in the current study was examined using the Folin-Ciocalteu method, which is affected by protein, ascorbic acid and reducing sugars, particularly fructose. Therefore, the higher sugar content in DPF may have influenced the TPC results to some extent Al Farsi et al. (2005).

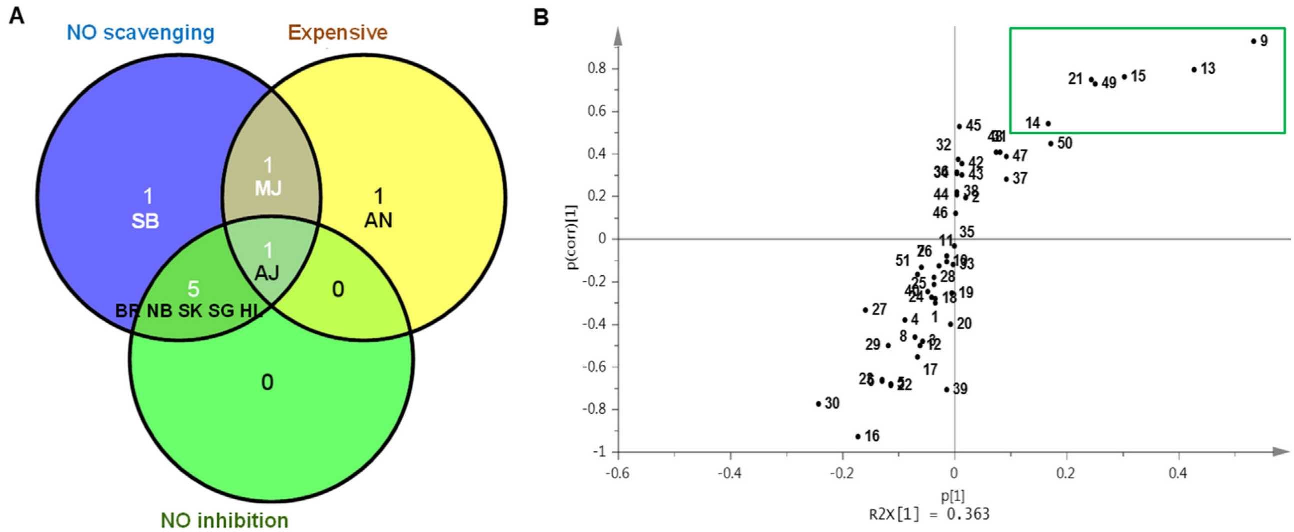

Fig. 2. The correlation between the marketed price of Saudi dates and NO scavenging/inhibition activities as illustrated by (A) Venn diagram (B) The S-plot resulting from OPLS-DA analysis modelling the Ajwa (AJ) dates against Anbara (AN). Among the prominent metabolites were those spotted in the green framed rectangle; which comprised of cysteine (14), GABA (21), sucrose (49), glycine (15), serine (13) and lysine (9).

Table 2
The biological activities and TPC of Saudi date palm fruit varieties.

Samples	NO		DPPH inhibition (%) (5 mg/mL)	TPC (mg GAE/g dw sample) (1.5 mg/mL)
	NO inhibition (%) (5 mg/mL)	NO scavenging (%) (5 mg/mL)		
BR	61.5 ± 2.2^{b}	65.0 ± 0.5^{a}	29.6 ± 2.7^{b}	3.35 ± 0.02^{b}
NB	59.3 ± 0.9^{b}	53.4 ± 1.1^{c}	36.7 ± 1.4^{d}	3.28 ± 0.03^{c}
SK	61.6 ± 1.4^{b}	61.1 ± 0.7^{d}	22.7 ± 1.9^{e}	3.26 ± 0.01^{c}
SG	65.5 ± 0.8^{a}	50.0 ± 0.5^{c}	30.2 ± 2.0^{b}	3.31 ± 0.01^{c}
HL	56.0 ± 1.9^{d}	53.4 ± 0.4^{c}	30.7 ± 1.7^{b}	3.34 ± 0.06^{b}
AJ	66.3 ± 1.7^{a}	66.2 ± 0.3^{a}	26.6 ± 1.9^{a}	3.25 ± 0.02^{a}
AN	15.9 ± 3.5^{b}	43.8 ± 1.9^{b}	31.6 ± 1.4^{b}	3.24 ± 0.01^{a}
MJ	22.6 ± 2.4^{e}	59.5 ± 1.0^{d}	40.0 ± 1.5^{c}	3.30 ± 0.07^{c}
SB	49.3 ± 2.6^{f}	59.8 ± 0.5^{d}	30.7 ± 1.4^{b}	3.31 ± 0.02^{c}

[a] Value = mean ± SD. Value in the same column with different superscript letter differ significantly at 5% probability level.

3.6. NO inhibitory/scavenging activities

The NO inhibiting capacity shown by DPF was evaluated as illustrated in Table 2. The ranking of the NO inhibition capacity, in descending order, was: AJ > SG > SK > BR > HL > NB > SB > MJ > AN. The percentages of inhibition by AJ and SG were not significantly different. The positive control, curcumin, inhibited the generation of NO with 97.0% inhibition at 100 μg/mL.

However, a different trend was observed for the NO scavenging activity. AJ consistently had the highest percentage, but the value was not significantly different from that of BR with respect to the NO scavenging. However, SG demonstrated the least inhibition of NO scavenging. Gallic acid, the positive control used for the analysis, showed an NO scavenging ability of 71.0% at 100 μg/mL. Differences in the antioxidant versus the scavenging inhibition and anti-inflammatory properties of different varieties of DPF were also reported by Tsai et al. (2007). Surprisingly, HL, which had the highest TPC, demonstrated moderate potency in both NO inhibitory/scavenging activities. Thus, it can be proposed that the phenolic compounds in these dates negatively influence the tested biological activities. Overall, the Saudi DPF demonstrated better NO scavenging capacity than for NO inhibition. A Venn diagram was developed to clarify the pattern of the distribution of selected DPF with biological activities, with respect to the correlation between the price and the biological activities (Mhlongo et al., 2016). The clarification was only applied to the DPF that had NO scavenging/inhibitions of more than 50% because all the DPF demonstrated weak DPPH scavenging power. Five DPF shared features that corresponded to good NO scavenging/inhibition activities (Fig. 2A). Both SB and MJ appeared to be unique in having a contrast in the level of NO scavenging compared to the NO inhibition. Interestingly, AN dates, which are one of the more expensive Saudi DPF, showed little correlation to neither NO scavenging nor NO inhibition. Another finding worth noting was that only AJ exclusively shared these three features, high NO scavenging, NO inhibition and expensive. The examination of the correlation suggests that the marketed prices of DPF were not directly correlated with the NO scavenging /inhibition.

The AN and AJ consistently demonstrated the opposite behavior in all studied parameters including physical characteristics and biological activities, as well as in multivariate data analysis despite both being considered to be premium quality product and being marketed at the same price. The quality assessment was further strengthened by determining the metabolites that may influence the observed differences between AN and AJ.

Therefore, the analysis was focused on assessing the differences between the DPF with the highest NO scavenging/ inhibition, AJ and the least active one, AN. The metabolites that contributed towards the separation between these two varieties were determined using Orthogonal Partial Least Squares Analysis Discriminant Analysis (OPLS-DA). The PCA is presented as a graphical presentation that displays the similarities and differences among the DPF. The lack of the predictive capability in the PCA was thereupon supplemented with the OPLS-DA to facilitate the identification of discriminatory metabolites (Yang et al., 2013). The resulting OPLS-DA as shown in Fig. S3 in Supplementary indicated a goodness of fit with an R2Y cumulative value of 1 and goodness of prediction with a Q2 value of 0.95. The sum of predictive and orthogonal variation (R2Xcum) of 0.99 was attained. The significance of the model was also examined utilizing the *p*-value ($p < 0.05$) of the CV-ANOVA. The obtained results were statistically significant on the basis of the observed *p*-value of 0.01.

The S-plot is customarily applied to identify significant biomarkers that discriminate between two groups. In this part of study, the influential metabolites were highlighted by the OPLS-DA S-plot, in which only the relevant metabolites with the correlation p(corr) of $\geq$0.5 and covariance of $p1 \geq 0.05$ were selected. The contributions of the X-variable to the observed clustering pattern (co-variation) was signified by the p_1 axis whereas the p(corr) axis indicates the reliability of every X-variable in achieving the group separation (correlation) (Yang et al., 2013). Knowledge regarding the combination of the $p1$ and p(corr) values is one of the benefits offered by the S-plot in which the verification of variables with the highest correlation coefficient and the greatest impact to the model can be simplified (Yang et al., 2013). The variables that are found far out in the S-plot are typically statistically relevant. Six metabolites complied with the defined cut-off point; sucrose, serine, lysine, GABA, glycine and also cysteine (Fig. 2B). These metabolites played roles in the differences between the metabolite compositions of AJ and AN.

The contrasts in the NO scavenging/inhibition between AN and AJ were then determined by performing a heatmap analysis, which was automatically achieved using the MetaboAnalyst software (Fig. 3A). The results are visualized using red for high and green for low amounts (Xia et al., 2009). It can be seen that AJ, which exhibited higher activities contained lower levels of fructose, shikimic acid, alpha glucose, beta glucose, lysine, glycine, GABA, serine, sucrose, cysteine, and gallic acid. Meanwhile, the opposite trends of prevalence were demonstrated by AN, which exhibited the higher amount of these particular 11 metabolites as shown by the darker shades of red. It is also interesting to note that of these 11 metabolites, six of them coincided with the discriminating variables suggested by the S-plot. It also can be observed that the darker shades of red observed for AJ correlated with brighter green colors for AN and vice versa. These metabolites may contribute to the observed activities for AJ and AN.

The correlations among the biological activities in the two varieties of interest, AJ and AN, was extended by performing a random forest (RF) analysis. One of the advantages of using RF was the acquisition of the mean decrease accuracy (MDA) (Gromski et al., 2015). The MDA was achieved by permuting the values of elements and measuring the probability of error increment, randomly. Furthermore, the RF was robust to the over-fitting issue, and no scaling was needed preceding the analysis (Gromski et al., 2015). The metabolites were ranked using MDA, and the 15 metabolites most prominently associated with the variation in the observed activities between AJ and AN are shown in Fig. 3B. Of these 15 metabolites, three were in agreement with those metabolites that differentiated AJ and AN as previously identified by the OPLS-DA analysis.

3.7. Proximate analysis

The proximate analysis was only determined for the selected six varieties which were BR, HL, NB, SK, SG and AJ. These six varieties were chosen as representatives of the cheapest (BR and HL), the intermediate (NB, SK and SG) and also the expensive variety, AJ. The AJ was the only variety selected from the premium group based on the distinctiveness that it showed throughout the prior analysis in the current study. The proximate analysis was accomplished by assessing the amounts of moisture, ash, protein, lipid, fiber and carbohydrate in the DPF. These chemical properties of dates are of high importance in grading, processing, preservation and storage as reported by Parvin et al. (2015). Several previous

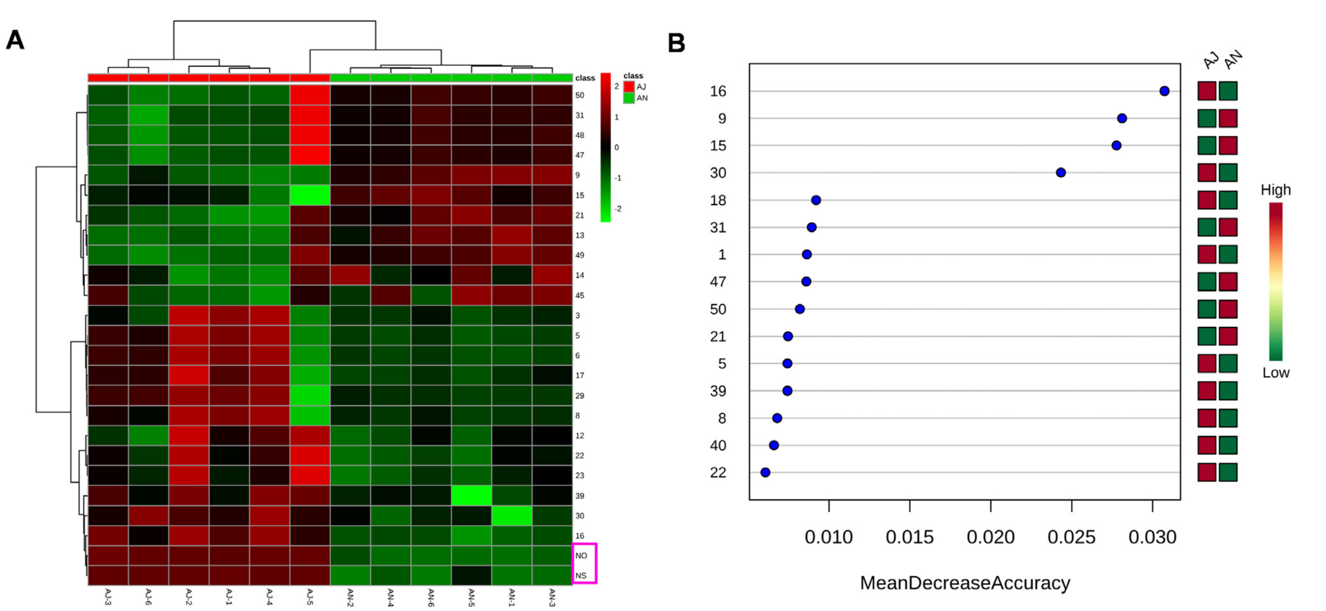

Fig. 3. The (A) heatmap analysis of the correlation between nitric oxide (NO) scavenging/inhibition activities and metabolite content of Ajwa (AJ) and Anbara (AN). The selected bio-activities were positioned in the purple framed rectangle; NO: NO inhibitory, NS: NO scavenging. The degree of variation was color coded, ranging from red (with positive value of 2) denoted as the highest to green (with negative value of -2), as the lowest abundances (B) The Mean Decrease Accuracy (MDA) of Random Forest analysis derived from comparison between AJ and AN dates.

studies have reported on proximate analyses of DPF. However, these studies emphasized different varieties of DPF from other sources including Bangladesh, Nigeria and Pakistan (Agboola and Adejumo, 2015; Assirey, 2015). The obtained results for the proximate composition of the selected six DPF were tabulated in Table 3.

The moisture level in food products is considered to be a critical aspect in preserving food from spoilage and thus affects the consumers' preferences (Agboola and Adejumo, 2015). The AJ dates had the highest moisture content, 28.6% whereas BR displayed the lowest level of moisture; 12.6%. Lower moisture content implies a longer shelf life but resulting in the development of dry dates, which are less preferred by the consumers (Assirey, 2015). This may be one of the reasons for the high preferences of consumers for AJ. According to Toutain (1967), the DPF were categorized into three groups; soft, semi-soft and dry dates based on the moisture content. The moisture content of the soft dates is normally at 30% or more. DPF with the moisture level in the range of 10% to 30% belong to the semi-soft date group, and dates are considered to be dry if the amount is lower than 10% (Habib and Ibrahim, 2011). Hence, it can be proposed that all these six varieties of DPF were the semi-soft dates.

The ash content serves as an index to the nutritive value of food. The ash content of these six varieties of dates ranged from approximately 5.23% for AJ to 6.20% for BR. The ash content in these DPF was higher than the range of ash content recorded for legumes which ranged from 4.4 to 5.0% (Agboola and Adejumo, 2015). With reference to the fat content, HL had the highest fat level which was significantly different from the rest of varieties. The BR, NB, AJ, HL and SK varieties may be classified as having a low fat content since the observed values were below 1.0% which was consistent of the fat content in Sudanese dates (Suleiman et al., 2011).

With respect to crude protein content, dates are not considered to be good sources of protein (Agboola and Adejumo, 2015). The protein content in these dates ranged from 2.08% to 3.1%. No significant differences among AJ, BR and SK but the differences among HL, NB and SG were significant. The results showed that AJ demonstrated the highest protein content at 3.1%. The highest value for crude fiber, 3.36% was observed in AJ. There were no significant variations among the AJ, BR, HL and NB varieties of dates, but the results were statistically significant varied between SG and SK dates. The percentages of fiber in the dates decreased in the following order AJ > BR > HL > NB > SG > SK. The fiber intake can facilitate digestion and absorption in the small intestine (Agboola and Adejumo, 2015). Carbohydrates, the major energy supply, contributed mainly to the mass in all varieties of Saudi DPF. The carbohydrate content was the highest in HL (76.8%) whereas the lowest amount of carbohydrate was in AJ (59.6%). The results were in the same range as those shown by three varieties in the study by Jamil et al. (2010). This further clarifies the varietal effects on the proximate composition of DPF. Carbohydrates in dates provide essential calories in the human diet (Agboola and Adejumo, 2015).

Similarly, the energy content was also investigated in this study, and results increased from 254 kcal for AJ to the highest energy content in HL with 318 kcal. The energy values completely

Table 3
Proximate composition of Saudi date palm fruit varieties.

Different varieties of date palm fruits							
Samples	Moisture	Ash	Fat	Protein	Fiber	Carbohydrate	Energy/100 g (kcal)
AJ	28.6 ± 1.3^{a}	5.23 ± 0.02^{c}	0.31 ± 0.04^{c}	3.1 ± 0.1^{a}	3.4 ± 0.2^{a}	59.6	254
BR	12.6 ± 0.1^{d}	6.20 ± 0.09^{a}	0.46 ± 0.18^{c}	3.0 ± 0.1^{a}	3.1 ± 0.1^{a}	74.4	313
HL	12.3 ± 0.2^{d}	5.42 ± 0.10^{bc}	7.33 ± 0.33^{b}	2.1 ± 0.2^{b}	3.1 ± 0.2^{a}	76.8	318
NB	21.7 ± 0.3^{b}	5.67 ± 0.15^{b}	0.44 ± 0.32^{c}	2.1 ± 0.2^{b}	3.1 ± 0.3^{a}	67.0	280
SG	15.6 ± 0.9^{c}	6.14 ± 0.13^{a}	1.89 ± 0.32^{a}	2.5 ± 0.1^{b}	2.2 ± 0.2^{b}	71.8	313
SK	23.3 ± 0.2^{b}	6.09 ± 0.03^{a}	0.08 ± 0.01^{d}	2.9 ± 0.2^{a}	1.9 ± 0.3^{b}	65.7	275

[a] Value = mean ± SD. Value in the same column with different superscript letter differ significantly at 5% probability level.

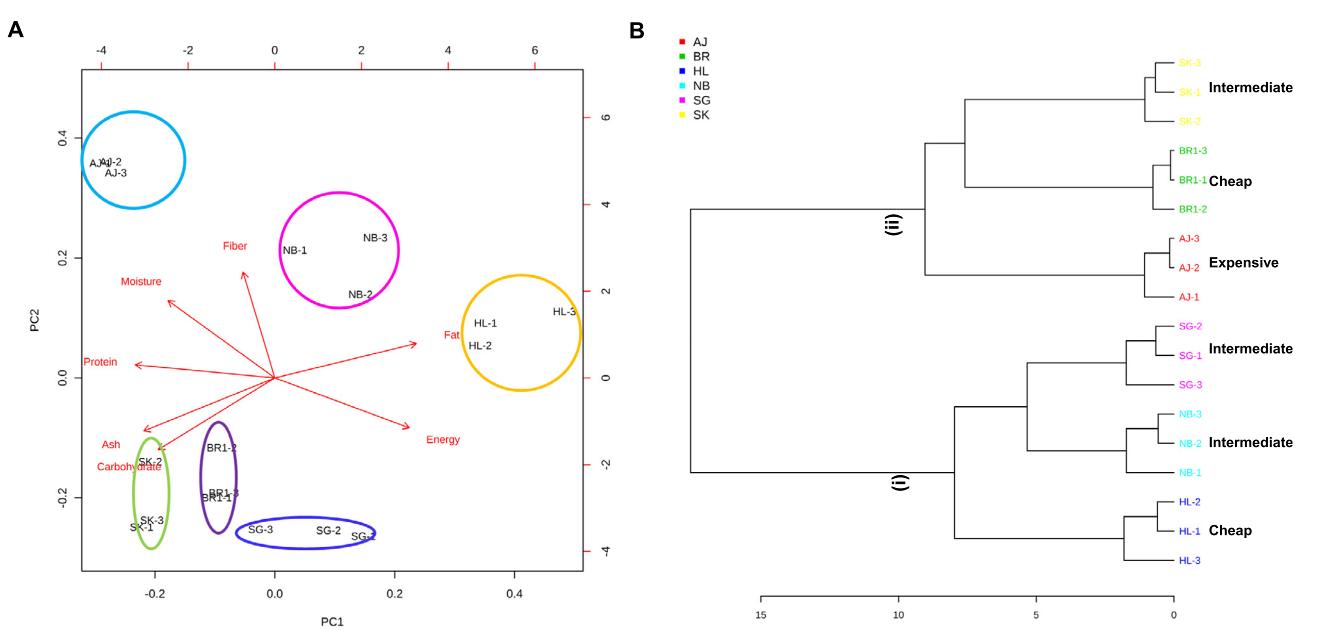

Fig. 4. The principal component analysis (PCA) of different varieties of Saudi dates highlighting variation in the amount of fiber, moisture, protein, fat, energy, ash and carbohydrates. The (A) PCA bi-plot and (B) hierarchical clustering analysis (HCA) dendrogram generated based on the proximate analysis data.

depended on the amount of dry matter, fats and carbohydrates. Therefore, the higher energy value observed for HL was due to the greater amounts of fat and carbohydrate shown by this variety. Generally, the obtained findings were consistent with the previous studies with some variations. Any differences might result from effect of the varieties, cultivation practices and environmental conditions (Gromski et al., 2015).

To obtain an overview of the principal variations among the applied proximate analysis, PCA was performed. Based on the PCA biplot (Fig. 4A), it can be observed that of the six DPF varieties, AJ, NB and HL were distantly clustered from the SK, BR and SG. AJ was distinguished from the rest of the DPF by moisture, fiber and protein. Meanwhile, HL which was among the cheapest DPF dates was separated by the fat content and energy level. The higher content of fiber in AJ would be beneficial to the digestion system whereas the high moisture content indicated the softness of the AJ dates, which might be one of the unique features of this variety.

In addition to the PCA, the grouping arrangement can be further explored by evaluating either the distance or the similarity among the DPF varieties by developing the hierarchical clustering analysis (HCA) dendogram. As presented in Fig. 4B, two distinct major clusters were observed; which were designated as group (i) and group (ii). It was interesting to note that there was no distinct similarity shared by the two cheapest varieties, HL and BR. The same trend was observed for the expensive AJ dates since there was no exceptional cluster can be observed for this premium DPF variety. Each cluster consisted of DPF varieties from all groups, cheap to expensive.

4. Conclusions

In conclusion, the AJ was the smallest, shortest in length and darkest colored Saudi DPF compared to the rest of the varieties. Nonetheless, the PCA score plot was not able to differentiate DPF according to the market price, suggesting that the varieties predominate over the price range. Another striking observation was that the expensive variety, AJ, and the cheapest one, HL clustered tightly in upper left quadrant of the PC1. All of the DPF varieties demonstrated less than 50% inhibition in the DPPH assay. HL showed the highest TPC, whereas AJ had the lowest TPC. With regards to the NO scavenging/inhibition activities, AJ had the highest activities. Visualization using the Venn diagram suggested that the marketed prices of DPF were not directly correlated with NO scavenging /inhibition. The supplemental analysis performed to compare AJ and AN via OPLS-DA analysis implied that sucrose, serine, lysine, GABA, glycine and also cysteine were the discriminatory metabolites for the consistent and interesting differences between these two varieties. Opposite trends of the relative ratio of metabolite abundance between AJ and AN were observed from the heatmap analysis. HL had a distinct high fat content, whereas AJ had the highest moisture content as revealed by proximate analysis. HCA analysis also indicated that there was no correlation between the marketed price and quality of DPF. With respect to the evidence provided by this study, it is plausible to propose that there was no recognizable correlation between the price and types of DPF varieties in Saudi as characterized by the variation in the physical features, biological activities, metabolites content or proximate analysis.

Conflict of interest

No conflict of interest is declared.

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