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Cluster analysis of antioxidant compounds in dates (*Phoenix dactylifera*): Effect of long-term cold storage

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ABSTRACT

Both soft (SD, Bam) and dry (DD, Kharak) date varieties were stored at 4 °C for six months followed by an additional one week storage at 18 °C. Antioxidant compounds (total phenolic content (TPC) and total flavonoid content (TFC)) of the dates increased following storage. Cluster analysis applied on TPC and TFC data before and after storage obtained two statistically significant clusters of SD and DD indicating that TPC and TFC had different behaviors according to the types of dates. As antioxidant concentration in dates was dependent on type of dates, selection of variety with high antioxidant compounds may be recommended. Further cold storage of up to six months followed by one week storage at 18 °C may further improve the level of antioxidant compounds.

1. Introduction

The fruit of the date palm (*Pheonix dactylifera*) is an important commercial crop in the Middle Eastern countries. Dates are a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium (Anwar-Shinwari, 1987; Gamil-Abdel-Hafez, Fouad-Shalaby, & Akhal, 1980). Besides nutritional value, date fruits are rich in phenolic compounds possessing antioxidant activity. Several studies have reported such activity of date fruits from Algeria (Mansouri, Embarek, Kokkalou, & Kefalas, 2005), Kuwait (Vayalil, 2002), Oman (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005a, 2005b), Iran (Biglari, AlKarkhi, & Easa, 2008), Bahrain (Allaith, 2008) and the USA (Vinson, Zubik, Bose, Samman, & Proch, 2005). These studies showed that fresh and dried dates varied quantitatively and qualitatively in their phenolic acids content.

Our interest has been the study of antioxidant compounds in Iranian dates. Therefore antioxidant activity, total phenolic content and total flavonoid content of eight selected date fruits from Iran have been reported in our previous study (Biglari et al., 2008). It was the phenolic compounds and flavonoids that gave rise to antioxidant activities in dates (Mansouri et al., 2005). It is of interest to note that several studies had indicated a change in the phenolic compounds profile of different fruits and vegetables upon chilled storage (i.e., 4 °C) (Kevers et al., 2007; Leja, Mareczek, & Ben, 2003; Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008). This observation prompted us to use statistical techniques to analyze antioxidant compounds in dates that change as a result of cold storage.

Cluster analysis (CA) is a multivariate technique, with the primary purpose of classifying the objects of the system into categories or clusters based on their similarities, and the objective is to find an optimal grouping for which the observations or objects within each cluster are similar, but the clusters are dissimilar to each other (Richard & Dean, 2002). Hierarchical clustering is the most common approach in which clusters are formed sequentially. The most similar objects are first grouped, and these initial groups are merged according to their similarities. Eventually as the similarity decreases all subgroups are fused into a single cluster. In the single linkage method, the distances or similarities between two clusters A and B is defined as the minimum distance between a point in A and a point in B (Richard & Dean, 2002):

$$D(\mathrm{A},\mathrm{B}) = \min\{d(y_i, y_j), \quad \text{for } y_i \text{ in A} \quad \text{and } y_j \text{ in B}\}, \tag{1}$$

where $d(y_i, y_j)$ is the Euclidean distance in (1).

At each step the distance is found for every pair of clusters and the two clusters with smallest distance (largest similarity) are merged. After two clusters are merged the procedure is repeated for the next step: the distances between all pairs of clusters are calculated again, and the pair with minimum distance is merged into a single cluster.

In relation to the importance of antioxidant compounds, the objectives of this research is to use CA to study the effect of long-term cold storage on antioxidant compounds in dates.

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2. Materials and methods

2.1. Plant material

Two types of dates were used for this study; a soft dates (SD) variety known as Bam dates that are grown mostly in Kerman Province and a dry date (DD) variety known as Kharak dates that are grown mostly in Fars Province of Iran (Biglari et al., 2008). The samples were selected identically in terms of size, colour and ripening stage. Each date weighed about 7–10 g per fruit. The dates were obtained from Tehran dates distribution centre at the beginning of 2006 harvest season and transported in a refrigerator in plastic containers. The harvest time of Kharak and Bam dates were November and August, respectively. It took eight days from the date of purchase, for the dates to be transported from Iran to Malaysia for the analyses and storage studies.

2.2. Chemicals and reagents

The chemicals and reagents used for analyzing the antioxidant compounds in dates were: gallic acid, catechin, sodium nitrate, sodium carbonate, Folin–Ciocalteu's phenol reagent and methanol were purchased from Merck (Darmstadt, Germany). All chemicals were of reagent grade.

2.3. Storage of dates

Dates from each variety were divided into three groups of 200 fruits, and each group of dates was placed inside a plastic container. One hundred dates from each container were removed for antioxidant compound analysis on the first day of storage. The containers were then closed with a plastic lid and stored in a refrigerator (Sharp, Japan) at 4 ± 2 °C for six months, followed by an additional one week storage at 18 °C (Leja et al., 2003; Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008). The stored dates (remaining 100 dates from each container) were analyzed for total phenolics and total flavonoids content.

2.4. Total phenolic (TPC) and total flavonoid content (TFC) analysis

The flesh part of date (100 g) was pitted, crushed and cut to small pieces with a sharp knife and dry-blended for 3 min with a blender (Panasonic, Penang, Malaysia). The extraction solvent was 300 ml methanol–water (4:1, v/v), and extraction carried out at room temperature (20 °C) for 5 h using an orbital shaker. The ratio of methanol and water used yielded the highest yield of phenolic compounds and flavonoids during preliminary trials. Similar ratio of methanol to water was used by Mansouri et al. (2005). Each date weighed about 7–10 g per fruit and for each extraction 100 g of each type of dates was used (Biglari et al., 2008). The extracts were then filtered and centrifuged (Hettich Zentrifugen) at 4000*g*, for 10 min and the supernatant was concentrated under reduced pressure at 40 °C for 3 h using a rotary evaporator (IKA-WERKE-RV06ML, Stanfer, Germany) to obtain date palm fruit (DPF) methanolic crude extract. The crude extract was kept in dark glass bottles for three days inside a freezer (Sanyo, Osaka, Japan) until use.

Analysis TPC and TFC were performed by following the methods described by Biglari et al. (2008). The TPC analysis was based on the methods described by Singleton and Rossi (1965), using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) and Folin Ciocalteau reagents. The optical density of the blue-coloured samples was measured at 765 nm. No information is available on the dominant phenolics compounds in Iranian dates, therefore the total phenolic contents were expressed as mg gallic acid equivalents (GAE)/100 g dry basis.

The determination of flavonoids was performed according to the colorimetric assay of Kim, Jeong, and Lee (2003) as described in our previous study (Biglari et al., 2008). No information is available on the dominant flavonoids compounds in Iranian dates, therefore a calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 g dry sample. Each sample was independently extracted in triplicate, and analyses were performed the same day.

2.5. Statistical analysis

2.5.1. Cluster analysis (CA)

Cluster analysis (CA) was performed according to the methods described by Richard and Dean (2002). The result of a hierarchical clustering procedure can be displayed graphically using a tree diagram, also known as a dendrogram, which shows all the steps in the hierarchical procedure (Alvin, 2002; Richard & Dean, 2002).

3. Result and discussion

Kharak dates are dry date with golden colour characteristics, while bam dates are soft dates that are dark brown in colour. After storage for six months Kharak dates lost firmness and tended to become more opaque. For bam dates, no colour change was observed, however sugar crystallization on the surface of the fruits was visually apparent.

Antioxidants activities based on ABTS and FRAP assays, total phenolics compounds (TPC) and total flavonoids compounds (TFC) of eight different varieties of dates from Iran have been compared (Biglari et al., 2008). A dry date (DD) variety (Kharak date, moisture content ~8%) stood out as the date with the highest level of antioxidant activity, TPC and TFC. A soft date (SD) variety (Bam date, moisture content 18–24%), on the other hand was one of the dates with the lowest antioxidant activities, TPC and TFC among the eight varieties of dates tested (Biglari et al., 2008). Studies on other fruits indicated the possibility of affecting the level of antioxidant compounds during storage (Kalt, 2005; Kevers et al., 2007). In contrast to many fruits that tend to lose stability over storage, dates are relatively stable over a long period of time if kept in a refrigerator. This prompted us to study the influence of low-temperature storage on antioxidant compounds in dates.

Descriptive statistics including the mean, maximum, minimum and standard deviation for TFC and TPC of SD and DD before and after storage are given in Table 1. Before storage the TPC and TFC of Kharak dates were higher than those of Bam dates. This confirms previous results reported on both types of dates (Biglari et al., 2008). Differences in antioxidant activities of dates are due to differences in variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions, and amount of sunlight received (Al-Farsi et al., 2007). In general, TPC of dates was higher than that of TFC. It can be seen that TFC in SD increased slightly over time of storage from zero to six months, whilst the difference is clear in dry dates (DD) since the content of TFC increased from 82.08 to 120.11 ((CEQ)/100 g dry sample). The difference in TPC in both types of dates is clear and the increment was high based on maximum and minimum contents. The spread around the mean was low in both types of dates and over both period for TFC, except for TPC in the period of six months that was slightly high. Both types of dates exhibited an increase in both TPC and TFC after six months of storage at 4 °C. The values of TPC and TFC of Kharak dates increased by ~36% and ~43%, respectively, while those of Bam dates increased by 223 and 28%, respectively. Several other studies have showed that fresh and dried dates from Algeria, Kuwait, Oman, Iran, Bahrain and USA varied quantitatively and qualitatively in their phenolic acids content

Table 1
Descriptive statistics of dates before and after storage at 4 °C for six months followed by seven days storage at 18 °C

Antioxidant compounds	Descriptive statistics	Types of date and storage times (months)[c]			
		SD		DD	
		0	6	0	6
TFC[a]	Minimum	2.56	3.50	82.08	120.11
	Maximum	3.06	3.66	87.94	123.53
	Mean	2.80	3.59	85.13	121.61
	Standard deviation	0.25	0.08	2.94	1.75
TPC[b]	Minimum	3.29	10.88	142.33	180.00
	Maximum	3.69	11.51	148.53	214.84
	Mean	3.49	11.30	145.70	197.41
	Standard deviation	0.20	0.36	3.14	17.42

[a] mg catechin equivalents (CEQ)/100 g dry sample.
[b] mg gallic acid equivalent (GAE)/100 g dry sample.
[c] Soft dates (SD), dry dates (DD).

and their antioxidant activity (Al-Farsi et al., 2005a, 2005b; Allaith, 2008; Biglari et al., 2008; Mansouri et al., 2005; Vayalil, 2002; Vinson et al., 2005). However, the increase in TPC and TFC of dates during storage has not been demonstrated. Such an effect has been shown to occur in other fruits, for instance Alasalvar, Al-Farsi, Quantick, Shahidi, and Wiktorowicz (2005) demonstrated that the total phenolics content of purple carrots and orange increased during storage that was related to the developmental changes and wound-like response. The response of plants to wounding may cause an increase of phenolic compounds that are involved in the repair of wound damage and in defence against microbial invasion (Dixon & Paiva, 1995).

The correlation between TFC and TPC was highly significant (0.99) ($P < 0.01$), indicating a strong positive relationship between them and that they showed similar behavior during storage. Fruits with high antioxidant capacity generally contain more antioxidant compounds and most of these has been showed to be phenolic compounds and in particular flavonoids (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002; Guo et al., 2003; Wang, Cao, & Prior, 1996). It is established that the antioxidant activity of dates was due mainly to the presence of water-soluble compounds with potent free radical-scavenging effects, including the phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones) (Biglari et al., 2008; Mansouri et al., 2005; Vayalil, 2002). Of all the antioxidative compounds present in Algerian dates, the cinnamic acid is the most dominant (Mansouri et al., 2005). Date varieties from different region of Oman had different levels and patterns of phenolic acids. Nine phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, and *o*-coumaric acid) have been tentatively identified. It was found that ferulic acid was the major phenolic acid for all date varieties in Oman (Al-Farsi, Morris, & Baron, 2006). The dominant antioxidative compounds in Iranian dates however have yet to be confirmed.

Cluster analysis (CA) was used to identify the similarity groups between the different types of dates during the two periods of time. CA rendered a dendrogram as shown in Fig. 1, grouping the two types of dates into two statistically significant clusters of soft dates (SD, Bam dates) and dry dates (DD, Kharak dates). This indicates that TFC and TPC have different behavior according to the type of dates. It can also be seen that within each cluster the difference between zero month and six months is clear since all observations under each time form a group which is different from another within the same type of dates. This grouping gives evidence that dates in each group have different characteristics from other types of dates and different content of TPC and TFC over time.

The effect of storage on antioxidants compounds is of interest since the storage treatment may be performed by dates' processors. Because dates are high in sugar and low in moisture, they are expected to be stable over an extended period of storage at

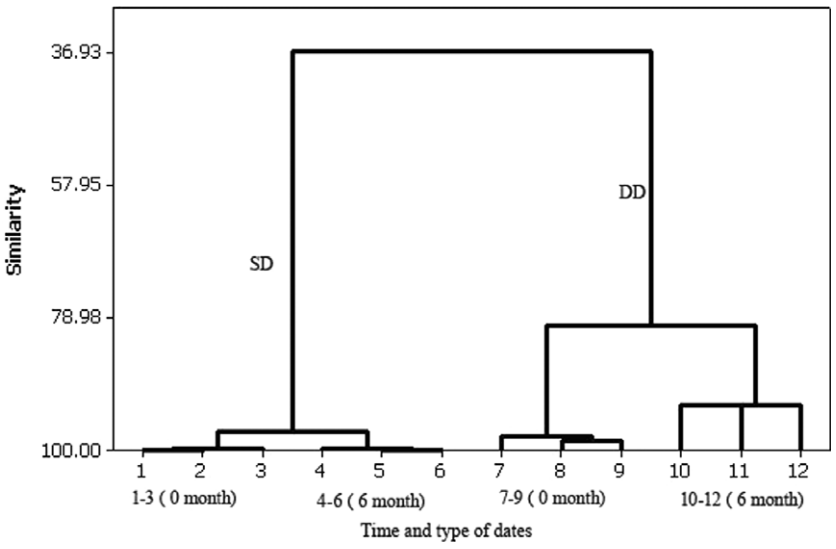

Fig. 1. Dendrogram showing clustering of dates over the two periods.

4 °C. The increase in the antioxidant compounds observed during cold storage of dates could well be due to ethylene action. This hormone stimulates activity of phenylalanine ammonia lyase, a key enzyme in biosynthesis of phenolic compounds and accumulation of phenolic constituents (Hwang, Myoung-Won, & Young-Hee, 1994; Ritenour, Ahrens, & Saltveit, 1995). Even though ascorbic acid was not measured in dates used for this study, it is possible to suggest that the contribution of total phenolics on antioxidant activity in dates is greater than that of ascorbic acid (Shivashankara, Isobe, Al-Haq, Takenaka, & Shina, 2004).

4. Conclusions

Total phenolics and total flavonoids of dates increased during long-term cold storage (4 °C) followed by an additional one week storage at 18 °C. The CA technique was useful in affording reliable classification of antioxidant contents in dates. In order to maximize antioxidant concentration, selected dates variety with a high initial antioxidant compounds should be kept in a refrigerator for up to six months. This may be processed into powdered products.

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