DOI:10.1021/jf901559a

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effects of Date (*Phoenix dactylifera* L., Medjool or Hallawi Variety) Consumption by Healthy Subjects on Serum Glucose and Lipid Levels and on Serum Oxidative Status: A Pilot Study

Wasseem Rock,[§,∥] Mira Rosenblat,[†,∥] Hamutal Borochov-Neori,[#,∥] Nina Volkova,[†] Sylvie Judeinstein,[#] Mazen Elias,[§] and Michael Aviram*,[†]

[†]The Lipid Research Laboratory, Rappaport Faculty of Medicine and the Rappaport Family Institute for Research in the Medical Sciences, Technion—Israel Institute of Technology, Haifa, Israel, [§]Internal Medicine C Department, Ha'Emek Medical Center, Afula, Israel, and [#]Southern Arava Research and Development, Hevel Eilot, Israel. [∥] Equal contribution.

The present pilot study analyzed, for the first time, the in vivo effect of Medjool or Hallawi date consumption by healthy subjects on serum glucose, lipids, and oxidative stress. Total phenolics concentration in the Hallawi versus Medjool dates was greater by 20−31%. The major proportion of the soluble phenolics in both date varieties consisted of phenolic acids, mainly ferulic acid and coumaric acid derivatives, and also chlorogenic and caffeic acid derivatives. Unlike the Medjool dates, Hallawi dates contained a significant proportion of catechins as well. In addition, both varieties contained a quercetin derivative. Both date varieties possess antioxidative properties in vitro, but the ferric ion reducing antioxidant power of Hallawi versus Medjool dates was higher by 24%. Ten healthy subjects consumed, for a period of 4 weeks 100 g/day of either Medjool or Hallawi dates. The date consumption did not significantly affect the subjects' body mass index (BMI), their serum total cholesterol, or their cholesterol levels in the VLDL, LDL, or HDL fractions. Most important, fasting serum glucose and triacylglycerol levels were not increased after consumption of either date variety, and serum triacylglycerol levels even significantly ($p < 0.05$) decreased, by 8 or 15% after Medjool or Hallawi date consumption, respectively. Basal serum oxidative status was significantly ($p < 0.01$) decreased by 33%, as compared to the levels observed before consumption, after Hallawi (but not Medjool) date consumption. Similarly, the susceptibility of serum to AAPH-induced lipid peroxidation decreased by 12%, but only after Hallawi date consumption. In agreement with the above results, serum activity of the HDL-associated antioxidant enzyme paraoxonase 1 (PON1) significantly increased, by 8%, after Hallawi date consumption. It is concluded that date consumption (and mainly the Hallawi variety) by healthy subjects, despite their high sugar content, demonstrates beneficial effects on serum triacylglycerol and oxidative stress and does not worsen serum glucose and lipid/lipoprotein patterns, and thus can be considered an antiatherogenic nutrient .

KEYWORDS: Dates; Medjool dates; Hallawi dates; oxidative stress; antioxidants; lipids; triacylglycerol

INTRODUCTION

Macrophage cholesterol accumulation and foam cell formation are the hallmarks of early atherogenesis (*1*), and oxidative stress has been shown to contribute to the development and progression of atherosclerosis (*2*−*4*). Indeed, low-density lipoprotein (LDL) can undergo oxidative modification by arterial macrophages (*2*), followed by an enhanced cellular uptake in the arterial wall, resulting in a significant increase in macrophage cholesterol and oxidized lipids accumulation (*2*). Flavonoids are the most potent nutritional antioxidants (*5*, *6*) as they inhibit LDL oxidation, secondary to their abilities to scavenge free radicals and to chelate transition metal ions (*7*, *8*).

Epidemiological studies have demonstrated an association between increased dietary intake of antioxidants from fruits, vegetables, tea, and wine, as well as vitamin E and vitamin C, and reduced morbidity and mortality from coronary artery disease (*9*, *10*).

We have demonstrated the antiatherogenic beneficial effects of fruit flavonoids consumption (licorice, grapes, pomegranate, marula) by atherosclerotic patients and also by healthy subjects (*11*−*15*).

Fruits of the date palm (*Phoenix dactylifera* L. Arecaceae) are an important component of the diet in the Middle East and North Africa. Dates are an ideal high-energy food as they have a high sugar content. They are also a good source of fibers and minerals,

*Address correspondence to this author at The Lipid Research Laboratory, Rambam Medical Center, Haifa 31096, Israel (e-mail aviram@tx.technion.ac.il; fax 972-4-8542130; telephone 972-4-8542970).

 Published on Web 08/14/2009

such as calcium, iron, magnesium, potassium, and zinc (*16*, *17*). The date fruit is used in folk medicine for the treatment of various infectious diseases and cancer (*18*), probably as a result of their immunomodulatory activity (*18*), antibacterial capacity (*19*), and antifungal property (*20*). Furthermore, aqueous extracts of dates were shown to have potent antioxidant activity (*21*), because they inhibit in vitro lipid and protein oxidation and possess free radical scavenging capacity. The above antioxidant activity is attributed to the wide range of phenolic compounds in the dates including *p*-coumaric, ferulic, and sinapic acids and flavonoids including procyanidins (*22*, *23*). It follows that dates may provide a significant source of daily dietary antioxidants in regions that consume significant quantities through traditional diets. On the other hand, the dates have high very high contents of sugars, which can potentially increase serum glucose and triacylglycerol levels, as well as serum oxidative stress and body mass index. However, no data are presently available on the effects of date consumption by healthy subjects on their serum glucose, lipids, and oxidative status. Thus, in the current study we examined, for the first time, the effect of regular consumption of dates from two varieties (Medjool and Hallawi) by healthy subjects on serum lipids profile, fasting blood glucose, and serum antioxidant properties.

MATERIALS AND METHODS

Throughout the study we used the Medjool or Hallawi date varieties, which were supplied shortly after harvest by HADIKLAIM, The Israeli Dates Growers/The Plants Production and Marketing Board.

Materials. Tannic acid, pyrogallol, catechin, epicatechin, catechin gallate, epicatechin gallate, gallocatechin, epigallocatechin, gallocatechin gallate, epigallocatechin gallate, naringin, *p*-coumaric acid, cinnamic acid, chlorogenic acid, caffeic acid, hydrocaffeic acid, ferulic acid, sinapic acid, sodium fluoride, phenyl acetate, thiobarbituric acid, tetramethoxypropane 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ), and Folin−Ciocalteu reagent were all purchased from Sigma-Aldrich Co. 2-Hydroxybenzoic (salicylic) acid, quercetin-3-β-glucoside, ellagic acid, and vitamin C were purchased from Fluka. 2,2′-Azobis(2-amidinopropane) hydrochloride (AAPH) was purchased from Wako, Japan. Acetonitrile HPLC grade was purchased from Merck and phosphoric acid from Frutarum, Israel. ^{3}H-Labeled cholesterol was purchased from Amersham. Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were purchased from Biological Industries, Beit Haemek, Israel.

Dates Processing. For the in vivo study both date varieties (Medjool and Hallawi) were washed with water and dried at 42 °C for 20 min. This was just a brief drying to remove the surface water after washing.

For water content and dry weight analyses, 10 g samples of thinly sliced date fruit were arranged in a single layer on drying plates and placed in a laboratory air-circulating oven (Heraeus Instruments, model UT6, Germany) set at 60 °C. After 24 h, the plates were weighed twice daily and removed from the oven after the same weight had been recorded in two consecutive measurements (up to a total of 48 h, depending on the amount of fruit and initial water content).

Moisture and dry weight contents were calculated as follows:

$$\text{water content } (\%) = \frac{\text{initial wt} - \text{final wt}}{\text{initial wt}} \times 100$$

$$\text{dry wt (DW, \%)} = \frac{\text{final wt}}{\text{initial wt}} \times 100$$

Date Mineral Analysis. Mineral content of date fruit tissue samples was analyzed by the analytical chemistry laboratory of the southern Arava Research and Development, a facility for plant, soil, and water chemical analysis certified by the Israeli Ministry of Agriculture Extension Service. Sodium, potassium, calcium, and magnesium were measured in oven-dried tissue digested to clarity with hot concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2, 30%). For iron, zinc, and manganese measurements, ash was prepared from oven-dried fruit tissue (5 h at 550 °C in a laboratory furnace, BIFA Laboratory Furnaces, model MS8-36) and digested to clarity with 6 N hydrochloric acid (HCl). Sodium and potassium were measured with a flame photometer (JENWAY, PFP7); calcium, magnesium, iron, zinc, and manganese were analyzed with an atomic absorption spectrometer (Perkin-Elmer, 3100).

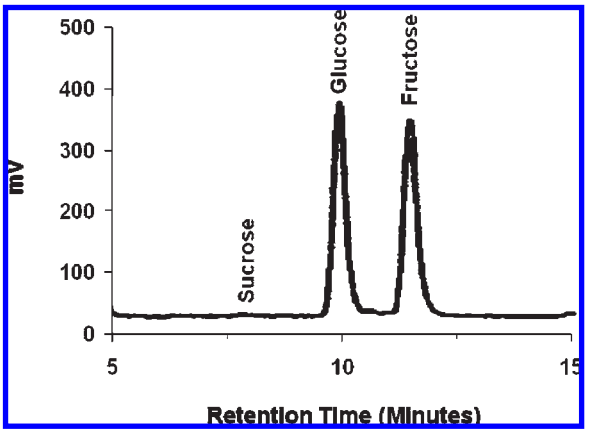

Figure 1. Typical HPLC chromatogram for Medjool date sugars. Date sugars were extracted with 80% ethanol, separated on a cation exchange column with DDW as the mobile phase, and detected with a refractive index (RI) detector.

Date Fiber Content. The fruit samples were analyzed for the content of nutritional fiber according to AOAC (Association of Official Analytical Chemists) Official Method 985.29, "Total Dietary Fiber in Foods—Enzymatic−Gravimetric Method". The analysis was carried out by Bactochem Ltd., Ness Ziona, Israel (laboratory for food and beverage chemical analysis accredited by the Israel Laboratory Accreditation Authority).

High-Pressure Liquid Chromatography (HPLC) Sugar Analysis. The protocol of Palevsky et al. (*24*) was employed. Mono- and disaccharides were extracted with ethanol from whole date fruit tissue (1:10 w/v at a final concentration of 80% ethanol). After centrifugation, the supernatant was filtered and the clear filtrate was dried under a stream of nitrogen. The dry pellet was dissolved in double-distilled water and kept at −20 °C until further analysis. The individual sugars were separated by HPLC using a Merck Hitachi LaChrom system composed of a pump L7100, column oven L7350 (set at 90 °C), and manual injector Rheodyne and equipped with a cation exchange column and precolumn (Merck, Polysphere CH CA) and a refractive index (RI) detector (Merck, LaChrom, L-7490). The mobile phase consisted of column-filtered water further distilled by a Corning Megapure System, MP-6A, and passed through a 0.20 μm nylon membrane. Highly pure sucrose, glucose, and fructose (HPLC grade, Sigma Chemical Co.) served as standards.

To measure sugar quantity, a four-point concentration−peak area calibration curve was constructed for each standard in the concentration range of 2−24 mg/mL. Date sugar solutions were diluted until values were within the calibration curve. Measurements were repeated four times for each sample.

A typical sugars HPLC chromatogram is shown in **Figure 1**.

Total Soluble Phenolics Content. Total soluble phenolics content (in tannic acid equivalents, or pyrogallol equivalents, mg/kg) was measured in methanolic extracts of whole fruit tissue samples. Fruit was sliced and thoroughly homogenized for 20 min on ice in double-distilled water (DDW, 1:2, w/v). The homogenized tissue was extracted in 80% methanol supplemented with 2 mmol/L sodium fluoride, at a ratio of 1:2 (w/v). After centrifugation (10000 rpm for 10 min at 4 °C, Sorvall Instruments RC5C, rotor no. SS-34), the clear supernatant was diluted with DDW (1:1, v/v) and the total phenolics were measured colorimetrically using Folin−Ciocalteu 2 N phenol reagent (*25*). Aliquots of 100 μL were added to 900 μL of reaction solution consisting of 200 μL of freshly prepared 10-fold diluted Folin−Ciocalteu reagent, 100 μL of Na_2CO_3, and 600 μL of DDW. Tannic acid or pyrogallol was used for the calibration curve (0−100 μg mL^{-1}). The absorbance at 765 nm was measured after 1 h of incubation, and the results, after subtraction of the contribution of glucose and fructose from the readings, were expressed in tannic acid or pyrogallol equivalents.

HPLC Partial Analysis of Soluble Phenolics in Date Fruit. Five grams of minced date fruit was homogenized with 10 mL of 80% methanol supplemented with 2 mmol/L NaF. The suspension was centrifuged (15000 rpm for 10 min at 4 °C, using Sorvall Instruments RC5C and rotor no. SS-34), and the supernatant was filtered through a 0.45 μm filter before injection. Samples of 20 μL were analyzed using the LaChrom Merck Hitachi HPLC system, consisting of pump L7100, column oven L7350, mixer−degasser L-7614, and manual injector Rheodyne, coupled with a diode array detector (DAD) with 3D feature (Multiwavelength Detector, Jasco MD-2010 Plus), interface (Jasco LC-Net II/ADC), and scientific software (EZChrom *Elite* Client/Server version 3.1.6 build 3.1.6.2433) that provides data acquisition in real time and post run data manipulation and integration facilities. An end-capped PurospherStar RP-18 column (250 × 4 mm LichroCART cartridge, 5 μm particle size) with an end-capped Lichrospher100 RP-18 guard column (4 × 4 mm LichroCART cartridge, 5 μm particle size) was used. The binary mobile phase consisted of phosphoric acid (0.1%, pH 2.4) (solution A) and acetonitrile (solution B). The system was run with the following gradient program: during the first 5 min, 85% solution A and 15% solution B; from 5 to 15 min, linear gradient of 85−0% solution A and 15−100% solution B; from 15 to 20 min, 100% solution B. A 10 min post run at initial conditions was carried out for column equilibration. Every run was monitored in real time by three display modes simultaneously: contour plot, chromatogram display at a chosen wavelength (usually 280 nm), and UV spectra. The oven temperature was set at 40 °C, and the pressure was 169 atm. Acetonitrile was of HPLC grade (LiChrosolv Merck); column-filtered water was further distilled by a Corning Megapure System, MP-6A, and passed through a 0.20 μm nylon membrane. Phosphoric acid and NaF were of analytical grade. A phenolics standard library was constructed as follows: Each standard (50−100 μg/mL methanol) was injected separately, and the data acquired by the photodiode array detector with the 3D feature were incorporated into the system phenolic standard library. The library was constructed from the following phenolics: catechin; epicatechin; catechin gallate; epicatechin gallate; gallocatechin; epigallocatechin; gallocatechin gallate; epigallocatechin gallate; naringin; *p*-coumaric, cinnamic, chlorogenic, caffeic, hydrocaffeic, ferulic, sinapic, and tannic acid; 2-hydroxybenzoic (salicylic) acid; quercetin-3-β-glucoside; and ellagic acid. Peak identification was performed by the software. Each peak was tested for purity by a three-point purity test and for similarity by a library search comparing the peak spectrum to that of the standards. A high similarity index and a common retention time with the standard are considered to be positive identification; a similar UV−vis absorption spectrum but a different retention time is considered to be a partial identification (e.g., derivative of the phenolic compound with the similar absorption spectrum). The software also calculates the peak area and the wavelengths of absorption maximum and minimum.

Date Free Radical Scavenging Capacity (DPPH Assay). DPPH is a radical-generating substance widely used to monitor the free radical scavenging abilities of various antioxidants (*26*). To analyze the free radical scavenging capacity of Medjool or Hallawi dates, similar volumes (0.2 mL) of the date methanolic extract (prepared as described above) were mixed with 1 mL of 0.1 mmol of DPPH/L in ethanol. The time course for the change in optical density at 517 nm was kinetically monitored for up to 320 s.

Date Ferric Reducing Antioxidant Power (FRAP). FRAP was measured by the colorimetric test originally developed to assess the FRAP of plasma (*27*). Clear methanolic extract was prepared as described earlier. Fifty microliters was added to 950 μL of freshly prepared FRAP working solution [50 mL of 300 mmol/L acetate buffer + 5 mL of 10 mmol/L 2,4,6-tripyridyl-*s*-triazine (TPTZ) + 5 mL of 20 mmol/L ferric chloride] in a 37 °C water bath. Absorbance at 593 nm was measured after 4 min. Vitamin C was used for the calibration curve (0−100 μg mL^{-1}), and the results were expressed in terms of vitamin C equivalents.

Subjects. Ten healthy subjects participated in the study (mean age = 36 ± 9 years, nonsmokers). The subjects consumed 100 g/day of Medjool variety dates for 4 weeks followed by a "washout" period of 4 weeks. At the end of the washout period, the same subjects consumed 100 g/day of Hallawi variety dates for an additional 4 weeks. Blood samples were collected before and after Medjool date consumption and before and after Hallawi date consumption. All blood samples were drawn between 7:00− and 8:00 a.m. after an overnight fast. Other measurements including body mass index (BMI), blood pressure, and liver and kidney function were performed at the same time intervals. The trial was conducted from September 2008 to January 2009. There was no possible seasonal change in the composition of the subject diets or habits, as the participants were directed not to perform any changes in their regular standard diet during the study. No change in systolic and diastolic blood pressure or in liver and kidney function during the study was noted (data not shown). All dates were stored during the study in home refrigerators (4 °C).

Table 1. Chemical Composition of Dates (Medjool vs Hallawi)[a]

chemical composition	Medjool dates	Hallawi dates
moisture[b]	27 ± 3	19 ± 2
potassium[c]	539 ± 54	525 ± 53
magnesium[c]	50 ± 5	75 ± 7
calcium[c]	54 ± 5	50 ± 5
iron[c]	0.83 ± 0.08	1.14 ± 0.11
zinc[c]	0.58 ± 0.06	0.61 ± 0.06
manganese[c]	0.36 ± 0.04	0.50 ± 0.05
sugars[c]	66400 ± 660	73600 ± 712
glucose[c]	32669 ± 295	37168 ± 327
fructose[c]	33731 ± 301	36432 ± 360
sucrose[c]		2208 ± 18
fibers[c]	7900 ± 72	7000 ± 65

[a] Sodium concentration was below detection. [b] Results are given as g/100 g. [c] Results are given as mg/100 g.

Serum Glucose and Lipids. Serum glucose was measured using an automated enzymatic UV hexokinase test (OSR 6521 Olympus). Total serum cholesterol was measured using an automated enzymatic color test (OSR 6516 Olympus). Serum HDL-cholesterol was measured using an automated enzymatic color test (OSR 6587 Olympus). Serum triacylglycerol was measured using an automated enzymatic color test (ORS 61118 Olympus). Serum LDL or VLDL levels were calculated. VLDL-cholesterol is calculated by dividing triacylglycerol by 5. LDL-cholesterol is calculated by subtracting (VLDL-cholesterol + HDL-cholesterol) from total cholesterol.

Serum Lipid Peroxidation. Basal serum oxidative status was determined by the thiobarbituric acid reactive substances (TBARS) assay, using tetramethoxypropane for the standard curve (*28*). For AAPH-induced serum lipid peroxidation, the serum samples were diluted ×4 with phosphate-buffered saline (PBS) and were incubated with 100 mmol/L AAPH for 2 h at 37 °C (*29*). The extent of lipid peroxidation was measured by the TBARS assay (*28*) or by the lipid peroxides assay (*30*).

FRAP. Thirty microliters of serum was mixed with 90 μL of H_2O. Freshly prepared FRAP reagent (900 μL) was warmed to 37 °C and added to the diluted samples. Absorbance readings were taken at zero time and after 4 min of incubation. Blank (120 μL of H_2O) and Fe^{II} standard solutions were tested in parallel (*27*).

Serum Paraoxonase 1 (PON1) Arylesterase Activity. Serum arylesterase activity was measured using phenyl acetate as the substrate (*31*). Initial rates of hydrolysis were determined spectrophotometrically at 270 nm. The assay mixture included 5 μL of serum, 1.0 mmol/L phenyl acetate, and 0.9 mmol/L $CaCl_2$ in 20 mmol/L Tris-HCl, pH 8.0. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The E_{270} for the reaction is 1310 M^{-1} cm^{-1}. One unit of arylesterase activity is equal to 1 μmol of phenyl acetate hydrolyzed per minute per milliliter.

Statistics. One-way ANOVA and Tukey post hoc means comparison tests were used to detect an overall effect of date consumption and to underscore the interactions (date variety × date consumption). The software used for this statistical analysis was Microsoft Excel multiple comparisons. Results are given as mean ± standard error of the mean (SEM).

RESULTS

In Vitro Studies. *Chemical Composition of Medjool and Hallawi Date Varieties*. The Medjool dates contained 27% water and 73% dry material, whereas the Hallawi dates contained 19% water and 81% dry material (**Table 1**). The chemical compositions of the dates employed in the study are shown in **Table 1**.

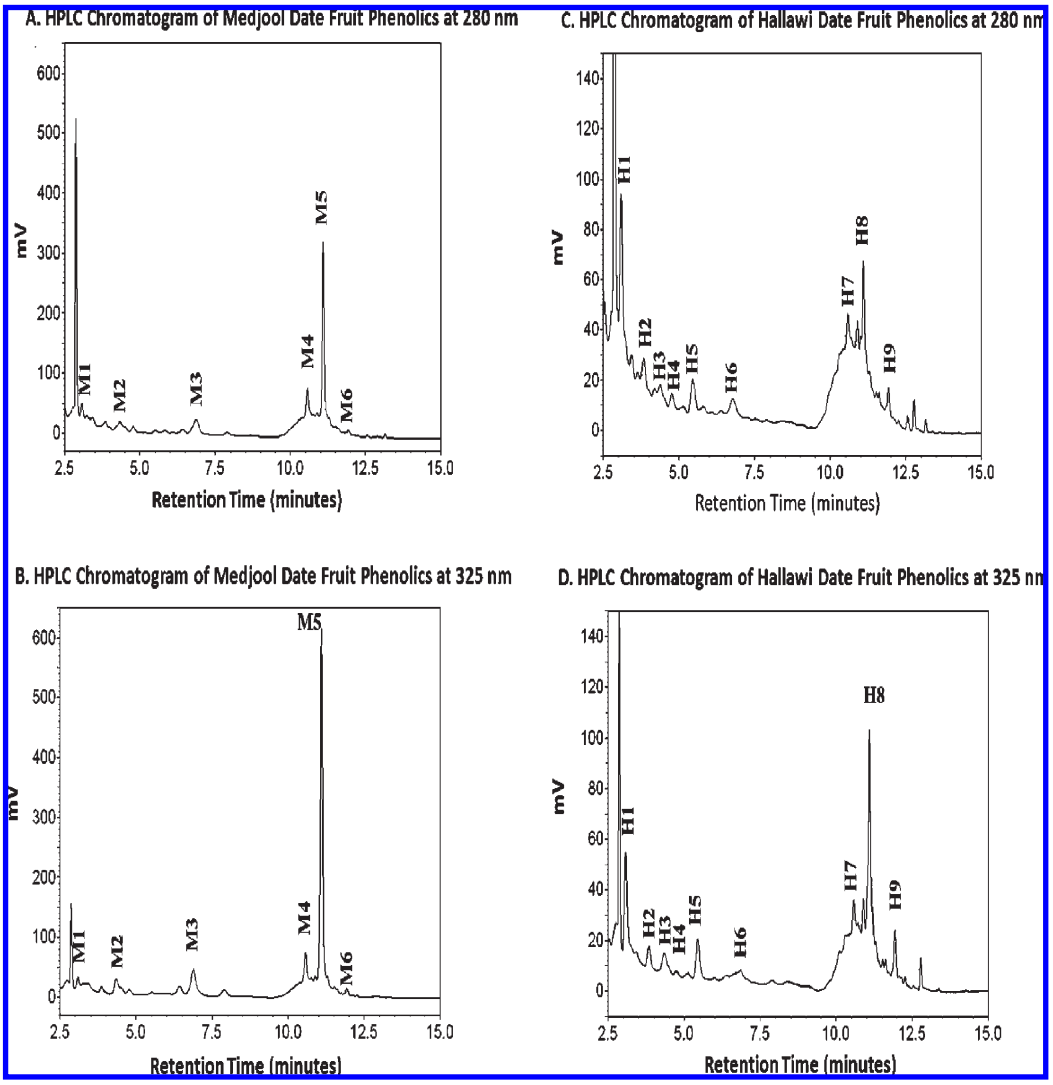

Figure 2. Phenolics composition of Medjool or Hallawi date variety as found by HPLC analysis of methanolic extracts of Medjool (**A**, **B**) and Hallawi (**C**, **D**) dates soluble phenolics performed as described under Materials and Methods: (**A**) representative chromatogram of all phenolics analysis of Medjool dates at 280 nm; (**B**) representative chromatogram of phenolic acids analysis of Medjool dates at 325 nm; (**C**) representative chromatogram of all phenolics analysis of Hallawi dates at 280 nm; (**D**) representative chromatogram of phenolic acids analysis of Hallawi dates at 325 nm. Peaks: M and H denote Medjool and Hallawi, respectively; M1 and H1, catechin derivative; H2, catechin derivative; M2 and H3, chlorogenic acid derivative; H4, catechin derivative; H5, caffeic acid derivative, M3, caffeic acid; H6, epicatechin derivative; M4 and H7, coumaric acid derivative; M5 and H8, ferulic acid; M6 and H9, quercetin derivative. mV represents millivolts.

Both date varieties are very rich in sugars, with similar concentrations of glucose and fructose. The Hallawi dates contain also a small amount of sucrose. Furthermore, they are rich in potassium, magnesium, and fibers (**Table 1**).

Phenolics Composition of Medjool or Hallawi Date Variety. Total phenolics concentration in the Hallawi dates versus Medjool date variety was significantly greater by 20% when measured as pyrogallol equivalents (619 ± 50 vs 517 ± 45 mg/kg, respectively). Similarly, total phenolics concentration in the Hallawi date versus Medjool date variety was significantly greater by 31% also when measured as tannic acid equivalents (1001 ± 70 vs 763 ± 45 mg/kg, respectively). Methanolic extracts of the Medjool or Hallawi date fruit were analyzed by HPLC to tentatively identify specific phenolics. Typical chromatograms of the original date extract at 280 (**Figure 2A**,**C**) and at 325 nm (**Figure 2B**,**D**) are presented. Comparable chromatograms were obtained with 2- and 3-fold dilutions of the extracts. The retention times in different runs varied within 0.05 min (RSD of 0.4−1.7%); peak area variation was within 0.5−3.5% (RSD).

On the basis of the standard library, constructed and utilized as described under Materials and Methods, the compounds corresponding to the major chromatogram peaks were tentatively identified or partially identified. The two date varieties differed considerably in their phenolics composition as revealed by the HPLC chromatograms; in the Medjool fruit mostly phenolic acids were detected, whereas in the Hallawi date chromatogram both phenolic acids and catechin derivatives were detected. In both varieties, the major phenolic acid was ferulic acid (peaks M5 and H8 in the chromatograms of Medjool and Hallawi, respectively). In addition, significant amounts of chlorogenic (peaks M2, H3), caffeic acid derivative (Hallawi) or caffeic acid (Medjool) (peaks M3 and H5), and coumaric acid derivatives (peaks M4 and H7) were detected. Unlike the Medjool date, the Hallawi fruit also contained a catechin derivative (peak H1) as a major component. Other catechin derivatives (peaks H2, H4, and H6) were also detected in the Hallawi cultivar. In both date varieties quercetin derivative was detected (M6 and H9).

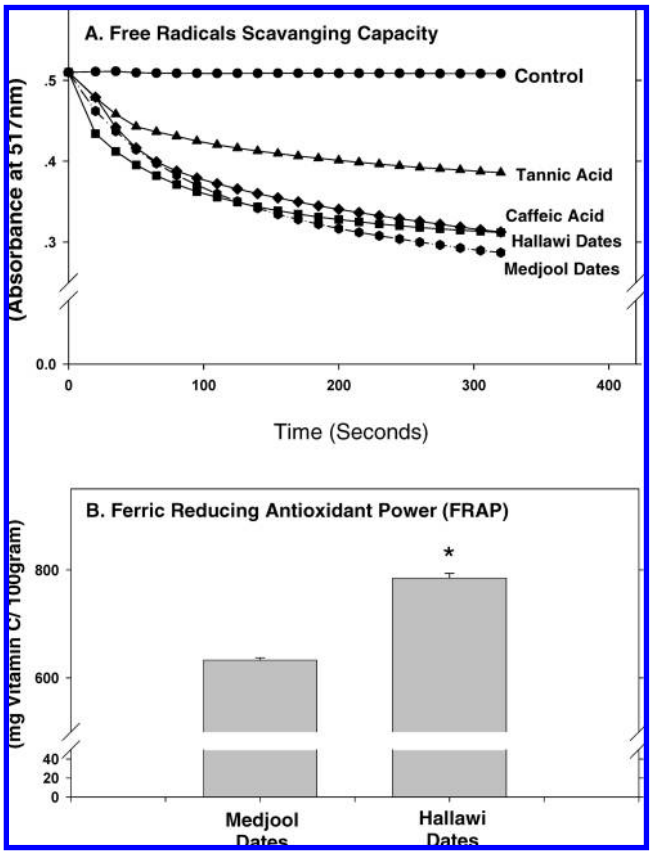

Figure 3. Antioxidative properties of Medjool or Hallawi date varieties: (**A**) free radical scavenging capacity of the date varieties determined using 0.1 mmol/L DPPH, as described under Materials and Methods [tannic acid or caffeic acid (0.01 mg/mL) was used for comparison; DPPH in the presence of 80% methanol served as the control; one representative experiment of three is shown]; (**B**) FRAP determined as described under Materials and Methods. Results are given as mean ± SEM of three different measurements.

Antioxidative Properties of the Medjool or Hallawi Date Variety. The antioxidative properties of both date varieties were first analyzed by their abilities to scavenge free radicals and to reduce ferric ions. Both the Medjool and Hallawi dates (0.01 mg/mL) demonstrated a potent antioxidant capability, as they both similarly decreased the absorbance of DPPH at 517 nm after 5.5 min of incubation by 44 or 39%, respectively (**Figure 3A**). For comparison, tannic or caffeic acids (0.01 mg/mL) decreased the DPPH absorbance by 21 or 39%, respectively (**Figure 3A**). Both date varieties were shown also to be potent antioxidants in the FRAP assay, but the Hallawi date antioxidant activity was greater by 24% as compared to the Medjool date FRAP (**Figure 3B**).

In Vivo Studies. Because the dates have, on the one hand, a high sugar content (which can possibly increase serum glucose and triacylglycerol levels, as well as enhance serum oxidative stress) but, on the other hand, possess in vitro antioxidative properties, we questioned the dates' in vivo effects on healthy subjects. In the present study, the effects of Medjool or Hallawi date consumption for 4 weeks, on serum glucose, lipid profile, and oxidative stress, were determined.

(*a*) *Effect of Medjool or Hallawi Date Consumption by Healthy Subjects on Serum Lipids and Glucose Concentrations*. BMI values were similar before and after 4 weeks of either Medjool or Hallawi dates consumption, despite the high calorie intake from these dates (**Table 2**). No significant changes in serum total cholesterol, LDL-cholesterol, or HDL-cholesterol could be noted after Medjool or Hallawi date consumption (**Table 2**). Serum triacylglycerol levels did not increase after 4 weeks of date consumption, but in fact decreased significantly (by 15%) with Hallawi dates (**Figure 4A**). Although the results did not reach significance at $p < 0.05$, the VLDL-cholesterol levels tended to be reduced after consumption of Medjool or Hallawi dates (by 8 or 15%, respectively, $0.1 > p > 0.05$, **Table 2**). Furthermore, and most important, serum glucose levels did not significantly increase after 4 weeks of Medjool or Hallawi date consumption (**Figure 4B**).

Table 2. Effect of Medjool or Hallawi Date Consumption by Healthy Subjects on Their BMI and on Serum Cholesterol and Lipoprotein Patterns[a]

date	BMI	total cholesterol	VLDL-cholesterol	LDL-cholesterol	HDL-cholesterol
Medjool, before	24 ± 1	194 ± 8	18.4 ± 2.0	122 ± 6	54 ± 5
Medjool,after	24 ± 1	200 ± 10	17.0 ± 1.8	124 ± 7	54 ± 3
Hallawi, before	24 ± 1	191 ± 9	20.2 ± 2.8	125 ± 4	56 ± 3
Hallawi, after	24 ± 1	195 ± 13	17.2 ± 1.8	123 ± 7	59 ± 4

[a] Data represent the results obtained before or after consumption of 100 g/day of dates for a period of 4 weeks. BMI, body mass index is given as (kg/m^2). Results for serum lipids are given in mg/dL, as mean ± SEM (n = 10).

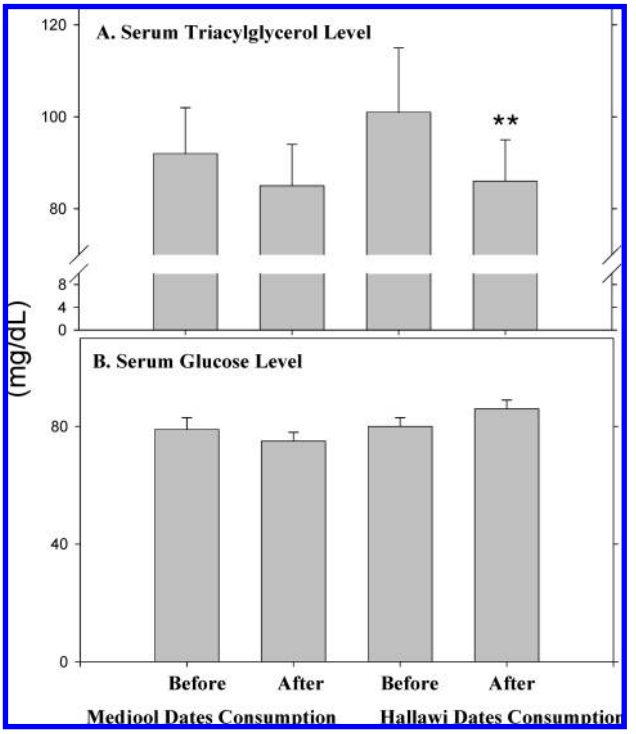

Figure 4. Effect of Medjool or Hallawi date consumption by healthy subjects on serum triacylglycerol and glucose levels: (**A**) serum triacylglycerol or (**B**) serum glucose levels measured as described under Materials and Methods. Results are given as mean ± SEM (n = 10). **, $p < 0.05$ after Hallawi date consumption versus before consumption.

(*b*) *Effect of Medjool or Hallawi Date Consumption by Healthy Subjects on Serum Oxidative Stress*. The basal serum oxidative status as measured by the TBARS assay was not significantly affected after 4 weeks of Medjool date consumption (**Figure 5A**). In contrast, 4 weeks of Hallawi date consumption resulted in a significant decrement in serum basal oxidative status, by 33%, as compared to the TBARS levels before Hallawi date consumption (**Figure 5A**). The susceptibility of serum to AAPH-induced serum lipid peroxidation, as measured by the TBARS (**Figure 5B**) or lipid peroxides assay (**Figure 5C**) was not affected by Medjool date consumption. In contrast, 4 weeks of Hallawi

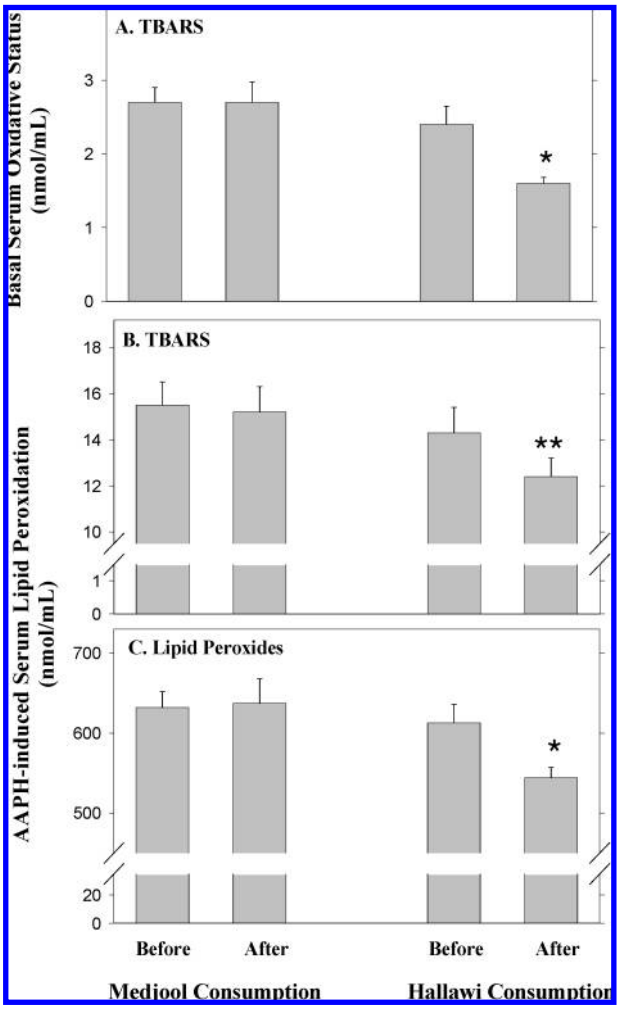

Figure 5. Effect of Medjool or Hallawi date consumption by healthy subjects on serum oxidative stress: (**A**) basal serum oxidative status analyzed by TBARS assay: (**B**, **C**) AAPH-induced serum lipid peroxidation analyzed by TBARS assay (**B**) or by lipid peroxides assay (**C**). Results are given as mean ± SEM ($n = 10$). ∗, $p < 0.01$ after Hallawi date consumption versus before consumption. ∗∗, $p < 0.05$ after Hallawi date consumption versus before consumption.

date consumption resulted in a significant decrement not only in basal serum oxidation (**Figure 5A**) but also in AAPH-induced serum lipid peroxidation by 13 or 11%, as measured by the TBARS (**Figure 5B**) or lipid peroxides (**Figure 5C**) assay, as compared to the levels obtained before date consumption.

(*c*) *Effect of Medjool or Hallawi Date Consumption by Healthy Subjects on Serum Antioxidants*. PON1 is an HDL-associated enzyme, which was shown to protect serum lipoproteins from oxidation (*32*). Thus, we analyzed the effect of the consumption of the two date varieties on serum PON1 activity. In accordance with the results obtained for serum oxidation, serum PON1 arylesterase activity was not affected by Medjool date consumption (**Figure 6A**), whereas Hallawi date consumption resulted in a significant increase, by 8%, in PON1 arylesterase activity, as compared to its activity before date consumption (**Figure 6A**). The FRAP of serum samples, however, was not affected either by Medjool date consumption or by Hallawi date consumption (**Figure 6B**).

DISCUSSION

In the current study we have demonstrated, for the first time, that date (Medjool or Hallawi variety) consumption by healthy

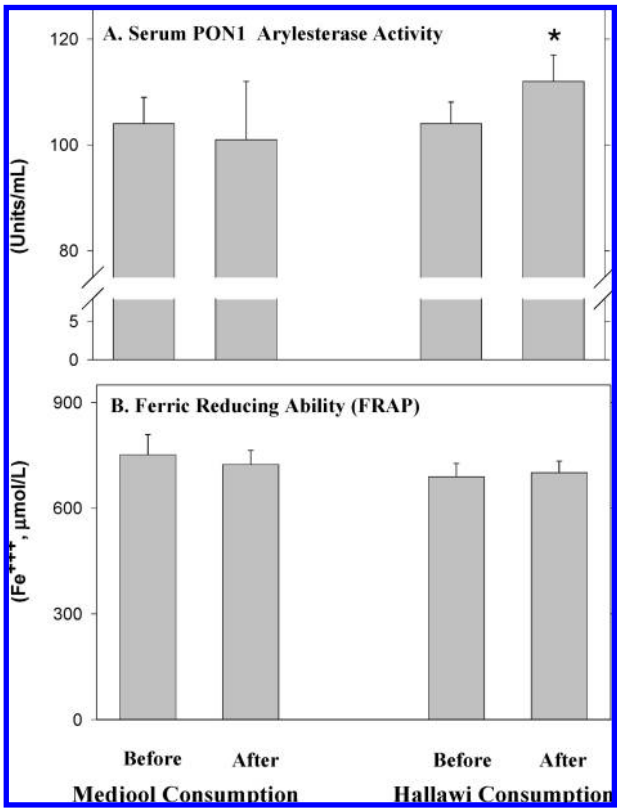

Figure 6. Effect of Medjool or Hallawi date consumption by healthy subjects on serum antioxidant capacity: (**A**) serum PON1 arylesterase activity or (**B**) serum FRAP determined as described under Materials and Methods. Results are given as mean ± SEM ($n = 10$). ∗, $p < 0.01$ after Hallawi date consumption versus before consumption.

subjects decreased serum triacylglycerol levels and that Hallawi date consumption also significantly reduced serum basal oxidative stress and increased PON1 activity. Neither date variety worsened serum glucose or lipoprotein patterns.

There are several studies, all performed in vitro, which demonstrate that dates possess antioxidant properties (*21*, *23*). The current study also demonstrated that the Medjool and Hallawi date varieties possess FRAP and the ability to scavenge free radicals. The antioxidant activity of dates could be attributed to the wide range of phenolic compounds in dates, which include *p*-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins (*22*, *23*). Indeed, in the current study, a partial HPLC analysis revealed that in Medjool dates, as well as in Hallawi dates, major soluble phenolics were ferulic acid and coumaric acid derivatives. In Hallawi dates, other significant components were catechin derivatives. In addition, in both date varieties chlorogenic and caffeic acid, as well as quercetin derivatives, were detected. It should be noted also that the date antioxidative properties could be also partially attributed to some unique sugars that were shown to be present in dates (*22*, *33*). Indeed, it was shown that the sugar fraction isolated from dates had the highest FRAP activity, as compared to other known refined sugars (*33*). Similarly, we have demonstrated that specific sugars in pomegranate juice, unlike grape sugars, can significantly decrease macrophages oxidative stress (*34*).

Consumption of Hallawi dates, but not of Medjool dates, by healthy subjects decreased basal serum oxidative stress, as well as the susceptibility of serum to AAPH-induced oxidation. The higher antioxidative properties of Hallawi versus Medjool dates in vivo could be related to the total phenolics concentration, which was higher in the Hallawi dates, and to the higher FRAP of

Hallawi dates versus Medjool dates. Furthermore, only the Hallawi dates (but not the Medjool dates) contain catechins, which are potent antioxidants. Another possibility is different absorption, metabolism, and bioactivity of the different phenolic compounds in the two varieties. In line with the above results, only Hallawi date consumption increased serum HDL-associated PON1 activity. PON1 was shown indeed to protect lipoproteins from oxidation (*32*, *6*). Furthermore, serum PON1 is inactivated under oxidative stress (*35*), and dietary antioxidants were shown to preserve its activity (*36*), as indeed was observed in the present study for the Hallawi dates that decreased serum oxidative stress and preserved serum PON1 activity.

Although consumption of the dates by healthy subjects can increase postprandial serum glucose concentration, it had no significant effect on fasting serum glucose levels. The decrement in serum triacylglycerol levels by both date varieties could be attributed, at least in part, to their high fiber concentration, as indeed it was shown that date waste dietary fibers decreased serum triacylglycerol levels in rats (*37*). Most of the date fibers are insoluble, and these fibers can easily bind to triacylglycerol in the intestine, followed by their secretion (*38*). High-fiber diets are recommended for diabetic and hypertriglyceridemic patients (*39*) and for reduction in the risk for cardiovascular diseases (*40*).

These latter observations could be related to the fact that serum cholesterol levels were not significantly affected by the consumption of either date variety and because the reductions in serum triacylglycerol levels and in serum oxidative stress were all relatively small over the month of date consumption.

We thus conclude that date consumption (and especially the Hallawi variety) by healthy subjects possesses beneficial antiatherogenic effects, as demonstrated by reduced serum triacylglycerol levels and oxidative status, and that date consumption does not worsen serum glucose levels.

ABBREVIATIONS USED

HPLC, high-pressure liquid chromatograph; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; BMI, body mass index; TBARS, thiobarbituric acid reactive substances; AAPH, 2,2′-azobis (2-amidinopropane) hydrochloride; FRAP, ferric reducing antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl; PON1, paraoxonase 1; SEM, standard error of the mean; DDW, double-distilled water; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum.

ACKNOWLEDGMENT

Dates for this research were obtained from HADIKLAIM, The Israeli Dates Growers/The Plants Production and Marketing Board.

LITERATURE CITED

(1) Lusis, A. J. Atherosclerosis. *Nature* **2000**, *404*, 233–241.
(2) Aviram, M.; Rosenblat, M. Oxidative stress in cardiovascular disease: role of oxidized lipoproteins in macrophage foam cell formation and atherosclerosis. In *Redox−Genome Interactions in Health and Disease*; Dekker: New York, 2003; Chapter 25, pp 557−590.
(3) Schulze, P. C.; Lee, R. T. Oxidative stress and atherosclerosis. *Curr. Arterioscler. Rep.* **2005**, *7*, 242–48.
(4) Heistad, D. D.; Wakisaka, Y.; Miller, J.; Chu, Y.; Pena-Silva, R. Novel aspects of oxidative stress in cardiovascular diseases. *Circ. J.* **2009**, *73*, 201–207.
(5) Kelawala, N. S.; Ananthanrayan, L. Antioxidant activity of selected foodstuffs. *Int. J. Food Nutr.* **2004**, *55*, 511–516.
(6) Aviram, M.; Kaplan, M.; Rosenblat, M.; Fuhrman, B. Dietary antioxidants and paraoxonases against LDL oxidation and atherosclerosis development. *Handb. Exp. Pharmacol.* **2005**, *170*, 263–300.
(7) Geleijnse, J. M.; Hollman, P Ch. Flavonoids and cardiovascular health: which compounds, what mechanisms? *Am. J. Clin. Nutr.* **2008**, *88*, 12–13.
(8) Vaya, J.; Aviram, M. Nutritional antioxidants: mechanisms of action, analyses of activities and medical applications. *Curr. Med. Chem.: Immunol. Endocr. Metab. Agents* **2001**, *1*, 99.
(9) Hooper, L.; Kroon, P. A.; Rimm, E. B.; Cohn, J. S.; Harvey, I.; Le Cornu, K. A.; Ryder, J. J.; Hall, W. L.; Cassidy, A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **2008**, *88*, 38–50.
(10) Kris-Etherton, P. M.; Keen, C. L. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Curr. Opin. Lipidol.* **2002**, *13*, 41–49.
(11) Rock, W.; Rosenblat, M.; Miller-Lotan, R.; Levy, A. P.; Elias, M.; Aviram, M. Consumption of wonderful variety pomegranate juice and extract by diabetic patients increases paraoxonase 1 association with high-density lipoprotein and stimulates its catalytic activities. *J. Agric. Food. Chem* **2008**, *56*, 8704–8713.
(12) Rosenblat, M.; Hayek, T.; Aviram, M. Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. *Atherosclerosis* **2006**, *187*, 363–371.
(13) Aviram, M.; Rosenblat, M.; Gaitini, D.; Nitecki, S.; Hoffman, A.; Dornfeld, L.; Volkova, N.; Presser, D.; Attias, J.; Liker, H.; Hayek, T. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin. Nutr* **2004**, *23*, 423–433.
(14) Fuhrman, B.; Volkova, N.; Kaplan, M.; Presser, D.; Attias, J.; Hayek, T.; Aviram, M. Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: increased resistance of LDL to atherogenic modifications, reduced plasma lipid levels, and decreased systolic blood pressure. *Nutrition* **2002**, *18*, 268–273.
(15) Borochov-Neori, H.; Judeinstein, S.; Greenberg, A.; Fuhrman, B.; Attias, J.; Volkova, N.; Hayek, T.; Aviram, M. Phenolic antioxidants and antiatherogenic effects of marula (*Sclerocarrya birrea* subsp. *caffra*) fruit juice in healthy humans. *J. Agric. Food Chem* **2008**, *56*, 9884–9891.
(16) Al-Shahib, W.; Marshall, R. J. The fruit of the date palm: its possible use as the best food for the future? *Int. J. Food Sci. Nutr.* **2003**, *54*, 247–259.
(17) Ali, A.; Al-Kindi, Y. S.; Al-Said, F. Chemical composition and glycemic index of three varieties of Omani dates. *Int. J. Food Sci. Nutr.* **2008**, *22*, 1–12.
(18) Puri, A.; Sahai, R.; Singh, K. L.; Saxena, R. P.; Tanton, J. S.; Saxena, K. C. Immunostimulant activity of dry fruits and plant materials used in Indian traditional medical system for mothers after child birth and invalids. *J. Ethnopharmacol.* **2000**, *71*, 89–92.
(19) Sallal, A.-K; Ashkenani, A. Effect of date extract on growth and spore germination of *Bacillus subtilis*. *Micrbios* **1989**, *59*, 203–210.
(20) Shraideh, Z. A.; Abu-El-Teen, K. H.; Sallal, A.-K. Ultrastructural effects of date extract on *Candida albicans*. *Mycopathologia* **1998**, *142*, 119–123.
(21) Vayalil, P. K. Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). *J. Agric. Food Chem* **2002**, *50*, 610–617.
(22) Hung, Y. J.; Tomas-Barberan, F. A.; Kader, A. A.; Mitchell, A. E. The flavonoid glycosides and procyanidin composition of Deglet Noor dates (*Phoenix dactylifera*). *J. Agric. Food Chem* **2006**, *54*, 2405–2411.
(23) Al-Farsi, M.; Alasalvar, C.; Morris, A.; Baron, M.; Shahidi, F. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *J. Agric. Food Chem* **2005**, *53*, 7592–7599.
(24) Palevsky, E.; Borochov-Neori, H.; Gerson, U. Population dynamics of *Oligonychus afrasiaticus* in the southern Arava Valley of Israel in relation to date fruit characteristics and climatic conditions. *Agric. For. Ent.* **2005**, *7*, 283–290.
(25) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(26) Blois, M. S. Antioxidant determination by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.
(27) Benzie, I. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.
(28) Buege, J. A.; Aust, S. D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302–310.
(29) Frei, B.; Stocker, R.; Ames, B. N. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9748–9752.
(30) El-Saadani, M.; Esterbauer, N.; El-Sayed, M.; Goher, M.; Nassar, A. Y.; Jurgens, G. Spectrophotometric assay for lipid peroxides in serum lipoproteins using commercially available reagent. *J. Lipid Res.* **1989**, *30*, 627–630.
(31) Gan, K. N.; Smolen, A; Eckerson, H. W.; La Du, B. N. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug. Metab. Dispos.* **1991**, *19*, 100–106.
(32) Ng, C. J.; Shih, D. M.; Hama, S. Y.; Villa, N; Navab, M; Reddy, S. T. The paraoxonase gene family and atherosclerosis. *Free Radical Biol. Med.* **2005**, *38*, 153–163.
(33) Phillips, K. M.; Carlsen, M. H.; Blomhoff, R. Total antioxidant content of alternatives to refined sugar. *J. Am. Diet. Assoc.* **2009**, *109*, 64–71.
(34) Rozenberg, O.; Howell, A.; Aviram, M. Pomegranate juice sugar fraction reduces macrophage oxidative stress, whereas white grape juice sugar fraction increases it. *Atherosclerosis* **2006**, *188*, 68–76.
(35) Aviram, M.; Rosenblat, M.; Billecke, S.; Erogul, J.; Sorenson, R.; Bisgaier, C. L.; Newton, R. S.; La Du, B. Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radical Biol. Med.* **1999**, *26*, 892–904.
(36) Aviram, M.; Kaplan, M.; Rosenblat, M.; Fuhrman, B. Dietary antioxidants and paraoxonases against LDL oxidation and atherosclerosis development. *Handb. Exp. Pharmacol.* **2005**, *170*, 263−300.
(37) Jwanny, E. W.; Rashad, M. M.; Moharib, S. A.; el Beih, N. M. Studies on date waste dietary fibers as hypolipidemic agent in rats. *Z. Ernaehrungswiss.* **1996**, *35*, 39–44.
(38) Kies, C. Non-soluble dietary fiber effects on lipid absorption and blood serum lipid patterns. *Lipids* **1985**, *20*, 802–807.
(39) Anderson, J. W. High-fiber diets for diabetic and hypertriglyceridemic patients. *CMA J.* **1980**, *123*, 975–979.
(40) Mozaffarian, D.; Kumanyika, S. K.; Lemaitre, R. N.; Olson, J. L.; Burke, G. L.; Siscovick, D. S. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. *JAMA−J. Am. Med. Assoc.* **2003**, *289*, 1659–1666.

Received February 4, 2009. Revised manuscript received July 6, 2009. Accepted July 31, 2009. Financial support for this research was obtained from HADIKLAIM, The Israeli Dates Growers/The Plants Production and Marketing Board.