Effect of date (*Phoenix dactylifera* L.) seed extract on stability of olive oil

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Abstract In this study, the antioxidant effect of date (*Phoenix dactylifera* L., Arecaceae) seed extracts at different concentrations (0.5 %, 1.0 % and 1.5 %) on the oxidative stability of olive oil at 60 °C was determined. Butylated hydroxyanisole (BHA) was used as positive control in the experiment. All extracts exhibited antioxidant activity compared to BHA up to 21 days. When antioxidant effect of extract concentrations were compared with BHA, the effect of 0.5 % extract concentration was more remarkable for olive oil up to 21 days. After 14 days of assay, all of seed extracts was effective at 60 °C in comparison with control. On the other hand, an important increase was observed in both the peroxide and free fatty acidity values during the experiment period. It concluded that date seed extract could be used as a oxidative inhibitor agent in oil and oil products.

Keywords Date seed extract · Oxidative stability · Olive oil · Fatty acids · Peroxide

Introduction

Antioxidants are compounds that can delay or inhibit the oxidation of lipid other molecules by preventing the initiation or propagation of oxidizing chain reactions (Velioglu et al. 1998). They can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation,

which is one of the major reasons for deterioration of food and pharmaceutical products during processing and storage (Halliwell 1997). Antioxidants commonly used in food products today are Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT). In recent years, the safety of synthetic food additives, including the possible toxicity of these chemicals used as antioxidants, has received increasing attention. So, there is need for other components to act as antioxidants and to render food products safer for mankind (Brannen 1975; Kahl and Kappus 1993; Özcan and Arslan 2011). Use of synthetic antioxidants to prevent free radical damage has been reported to involve toxic side effects (Faure et al. 1990; Feher and Pronai 1993; Cornwell et al. 1998), making attractive the search for antioxidant and scavenger natural compounds. Therefore, there is a growing interest on natural and safer antioxidants (Moure et al. 2001; Oktay et al. 2003). A need for identifying alternative natural and safe sources of food antioxidants has been created, and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (Özcan and Akgül 1995; Skerget et al. 2005; Özcan and Arslan 2011). So, the date palm is largely grown in arid and semiarid regions of the world. World production of date seeds reached approximately 863,000 t in 2004. Date seeds are being wasted in large quantities or used mainly for animal (camels, cattle, sheep and poultry) feed. In addition, date seeds are used on a very limited scale in making a caffeine-free beverage with a coffee-like flavour (Hussein et al. 1998; FAO 2004; Rahman et al. 2007; Habib and Ibrahim 2008; Habib and Ibrahim 2011). Studies have been increasingly showing that date seeds possess significant nutritional value, especially in terms of their fibre and antioxidant content (Habib and Ibrahim 2008). Date seeds have been shown to contain significant amounts of antioxidants (Al-Farsi and Lee 2008). Such as, hydroxytyrosol, protocatechuic acid, tyrosol, gallic acid, caffeic acid, *p*-coumaric acid and oleuropein (Aberoumand and Deokule 2004). So, in vivo studies on the activity of date

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seeds on stability of olive oil are lacking. Therefore, the purpose of present study was to determine the antioxidant effects of date seed extracts of the different concentrations on olive oil stored at 60 °C in darkness.

Materials & methods

Material

The seeds of date (*Phoenix dactylifera* L.) (sukary cv.) was provided from a local farm in Riyadh, Saudi Arabia, at the beginning of the 2010 harvest season. The seeds were washed to get rid of any adhering date flesh, and air-dried. Then they were further dried at about 50 °C for 5 h. Seeds were ground in grinder to pass 1–2 mm screens and then kept at 4 °C up to analysis.

Olive oil without adding any antioxidant was supplied by cool press processing. Synthetic antioxidant [Butylated hydroxyanisole (BHA)] (Sigma) was preferred because of its common usage by manufacturers of vegetable oil to retard the oxidation.

Extraction of date seeds

About 10 g of ground date seeds was shaken with 50 ml of methanol (Merck, Darmstadt, Germany) in a flask at °C 35 for 24 h. Solution with methanol was filtered by filter paper, and then was centrifuged. The solvent was evaporated by rotary evaporator. Different concentrations of residue were used as natural antioxidant in olive oil.

Evaluation of antioxidant activity

Analyses were conducted according to the AOCS (1990). A calculated quantity of the BHA (0.01 %) and extracts (0.5 %, 1.0 % and 1.5 %) according to olive oil amount in stored beherglass were added directly into olive oil at room temperature with the concentrations of 0.5 %, 1.0 % and 1.5 % and dissolution was obtained by manual shaking for about 5 min. A control sample was prepared under the same conditions without addition of any antioxidant. Fifty grams of each sample was stored in 8×10 cm beherglass and kept in oven at 60 °C in the dark. For the determination of peroxide number, a given weight of olive oil was dissolved in a mixture of acetic acid:chloroform (3:2, v/v), and saturated solution of KI (1 ml) was then added. The liberated iodine was titrated with a sodium thiosulphate solution (0.01 N) in the presence of starch as an indicator (AOCS 1990). For the free fatty acidity expressed as a percentage of oleic acid, a known weight of olive oil was dissolved in a mixture of diethyl ether/ethanol (1:1 v/v). The mixture was titrated with 0.1 N sodium hydroxide by using phenolphthalein as indicator.

Determination of fatty acids

Fatty acid compositions of olive oil were determined using the method described by Hişil (1998) with some modifications. The olive oil sample (50–100 mg) was converted to its fatty acid methyl esters (FAME). One drop of the oil was dissolved in 1 mL of *n*-heptane, 50 µg of sodium methylate was added, and the closed tube was agitated vigorously for 1 min at room temperature. After addition of 100 µL of water, the tube was centrifuged at 4,500 g for 10 min and the lower aqueous phase was removed. Then 50 µL of HCl (1 mol with methyl orange) was added, the solution was shortly mixed, and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added, and after centrifugation at 4,500 g for 10 min, the top *n*-heptane phase was transferred to a vial. The methyl esters of the fatty acids (1 µl) were analysed in a gas chromatography (Shimadzu GC-2010) equipped with a flame ionising detector (FID), a fused silica capillary column (60 m×0.25 mm i.d.; film thickness 0.20 mikrometere). It was operated under the following conditions: oven temperature program. 90 °C for 7 min. Raised to 240 °C at a rate 5 °C/min and than kept at 240 °C for 15 min; injector and detector temperatures, 260 and 260 °C; respectively, carrier gas. nitrogen at flow rate of 1.51 ml/min; split ratio. 1/50 µl/min.

A standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to identify sample peaks. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times (AOCS 1990). The peak areas were computed by the integration software, and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization. The results are mean values of three replicates.

Statistical analyses

Results of the research were analysed for statistical significance by analysis of variance (Püskülcü and İkiz 1989).

Results and discussion

Table 1 shows the data on antioxidant activity of date seed extracts. Date seed extract showed differences depending on used concentrations during experiment. On the 7 day of the assay, all the extracts exhibited antioxidant activity in varying degrees on olive oil. Antioxidant activities of date extracts were slightly weaker when compared to BHA added samples. Generally, clear antioxidant effect of extracts was not observed on olive oil until the 14th day of assay when it is seen that there are significant differences between all the oil, control sample, extracts added oil and levels of addition

Table 1 Changes on peroxide, free fatty acidity and viscosity values of olive oil added with date seed extract

Samples	7. day			14. day			21. day			28. day		
	Peroxide values(meq O ₂ /Kg)	Free fatty acid (Oleic %)	Viscosity (m.Pas)	Peroxide values(meq O ₂ /Kg)	Free fatty acid (Oleic %)	Viscosity (m.Pas)	Peroxide values(meq O ₂ /Kg)	Free fatty acid (Oleic %)	Viscosity (m.Pas)	Peroxide values(meq O ₂ /Kg)	Free fatty acid (Oleic %)	Viscosity (m.Pas)
BHA	8.37±0.98 ^a	3.62±0.21 ^a	50.30±2.13 ^a	9.06±0.36 ^a	3.54±0.17	53.27±1.38	9.84±0.17	3.54±0.08	36.73±1.13	12.49±0.17	3.81±0.11	36.07±0.69
0.5 %	8.05±1.02 ^a	3.55±0.36 ^a	50.90±1.56 ^a	8.84±0.72 ^b	3.45±0.81	50.73±1.57	12.10±0.21	3.08±0.03	41.50±1.21	25.12±0.21	3.88±0.09	42.83±1.09
1.0 %	7.70±0.78 ^{ab}	3.92±0.43 ^a	44.90±1.32 ^{ab}	8.59±0.78 ^b	3.49±0.33	53.37±1.74	15.00±0.43	3.45±0.11	35.90±1.78	26.60±0.13	3.66±0.13	40.97±0.83
1.5 %	7.96±0.56 ^{ab}	3.76±0.47 ^a	50.90±2.36 ^a	8.46±0.97 ^b	3.77±0.67	53.10±1.83	14.50±0.18	3.51±0.17	37.23±0.78	25.81±0.27	3.57±0.21	38.83±0.76
Control	6.60±0.73 ^c	3.73±0.56 ^a	45.80±1.09 ^{ab}	9.17±0.89 ^a	3.53±0.57	46.83±1.95	12.50±0.25	3.47±0.07	38.77±0.67	26.60±0.32	3.62±0.17	42.20±1.13

^a values are reported as mean±SD of three replications^b means within a column with different superscript are significantly different at $p<0.05$

($p<0.05$). On the other hand, while antioxidant activity increased together with extract concentration, it decreased at the same form after this period. All extracts exhibited antioxidant activity compared to BHA up to 21 days of experiment. When antioxidant effect of extract concentrations were compared with BHA, the effect of 0.5 % extract concentration was more remarkable for olive oil upto 21 days (Table 1). After 14 days of assay, all of seed extracts were effective at 60 °C in comparison with control. But when it was compared to BHA, the activity was at a very limited level. Significant differences ($p<0.05$) existed among different concentrations used, with some exception (Table 1). The results obtained suggest a protective effect of date seeds against in vivo oxidative damage, possibly through the action of their bioactive antioxidants (Habib and Ibrahim 2011). Total phenolic contents were reported by them to be for them about 220–520 mg/g oil respectively and the results showed that the date seed oil could be easily stored (Besbes et al. 2004a, b). Date seeds previously been examined for extractable high value-added components for incorporation into functional foods (Hamada et al. 2002). As mentioned before, *P. dactylifera* L., seeds could easily be conserved and used in cosmetics, pharmaceuticals and food products (Ardekani et al. 2010).

Generally, peroxide values, free fatty acidity and viscosity values of olive oil added date seed extracts at the different concentrations changed compared to control and BHA (Table 1). Free fatty acidity values of samples partly increased after 21th days of experiment. In addition, viscosity values of treated samples decreased after 14th days of experiment. According to these results, date seed extract inhibited the polymerization of olive oil at 60 °C. While viscosity values of samples were measured between 44.90 m.Pas (1 %) and 50.90 m.Pas (0.5 % and 1.5 %) at the first period of experiment, the values were established between 36.07 m.Pas (BHA) and 42.83 m.Pas (0.5 %) at the last period of experiment. The viscosity values differed significantly after date seed extracts addition ($p<0.05$). Several biological activities including antioxidant properties are usually assigned to the plant derivatives such as essential oil, oleoresin and extracts or to some of their constituents (Prakash 1990; Loo and Richard 1992).

The effect of date seed extracts on fatty acid composition of olive oil are given in Table 2. It is seen that there are significant differences between all the extract concentrations, control groups, extract concentrations and control samples ($p<0.05$). During the experiment, contents of palmitic, oleic, linoleic and linolenic acids of olive oil added with BHA decreased while arachidic acid content increased. Generally, oleic acid contents of samples during experiment ranged between 66.42 % (with 1.5 % extract) to 73.61 % (with 0.5 extract). At the last period of experiment, oleic acid content of samples partly decreased. Fatty acid content of samples exhibited differences depending on extract concentrations.

Table 2 Fatty acid composition of olive oil added with date seed extracts at 60 °C

Days and samples	Fatty acids (%)					
	Palmitic	Oleic	Linoleic	Linolenic	Arachidic	Total
7. days						
BHA	14.33±0.12a ^a	72.59±1.06	11.69±0.13ab	0.75±0.01	0.53±0.01	99.89
0.5 %	14.44±1.09a ^b	72.83±1.12	11.96±0.21ab	0.69±0.03	0.00±0.00	99.89
1.0 %	14.18±0.11a	71.79±0.99	11.72±0.08ab	0.70±0.08	0.55±0.03	98.94
1.5 %	14.26±0.07b	72.32±2.37	11.59±0.07	0.80±0.11	0.56±0.09	99.53
Control	14.34±0.23a	72.74±1.52	11.44±0.27ab	0.77±0.06	0.56±0.011	99.85
14. days						
BHA	14.30±0.03b	71.91±0.89ab	11.91±1.02b	0.69±0.03	0.55±0.06	99.36
0.5 %	14.49±0.05a	73.61±0.67a	9.97±0.08c	0.78±0.08	0.57±0.08	99.42
1.0 %	14.15±0.07b	71.49±1.02ab	11.44±0.13b	0.67±0.07	0.53±0.03	98.28
1.5 %	12.10±0.13c	66.42±1.34c	13.76±1.06a	0.00±0.00	0.00±0.00	92.28
Control	14.15±0.21b	70.89±2.13b	11.98±0.89b	0.66±0.05	0.53±0.03	98.21
21. days						
BHA	13.77±1.02b	69.65±1.21b	12.44±0.56a	0.63±0.02	0.62±0.11	97.11
0.5 %	14.08±1.07a	71.49±2.17ab	11.72±0.35b	0.58±0.06	0.34±0.01	98.21
1.0 %	14.44±0.78a	72.78±0.89a	11.47±0.09b	0.60±0.04	0.67±0.06	99.96
1.5 %	14.58±0.63a	72.67±0.76a	12.12±0.01b	0.61±0.03	0.00±0.00	99.98
Control	14.51±0.21a	72.64±1.14a	11.52±0.23b	0.58±0.07	0.72±0.12	99.97
28. days						
BHA	13.69±0.56b	69.60±0.61b	12.07±0.47b	0.62±0.01	0.80±0.09	96.78
0.5 %	14.36±0.22a	72.70±1.43a	11.20±0.32bc	0.49±0.03	0.47±0.03	99.22
1.0 %	14.51±0.27a	72.67±1.38a	11.10±0.17bc	0.52±0.05	0.00±0.00	98.80
1.5 %	14.72±0.42a	68.68±0.76c	16.06±1.29a	0.50±0.03	0.00±0.00	99.96
Control	14.53±0.87a	70.34±1.28ab	10.86±0.63	0.52±0.07	0.47±0.01	96.72

^a values are reported as mean±SD of three replications

^b means within a column with different superscript are significantly different at $p<0.05$

Total fatty acid contents of samples changed between 92.28 and 99.98 %. Date seed oil has been reported to be composed of about 44 % saturated fatty acids, 41 % monounsaturated fatty acids and 14 % polyunsaturated fatty acids (Besbes et al. 2004a).

Therefore, the biological activities of these extracts could provide a chemical basis for some of the health benefits claimed for *Phoenix dactylifera* L seeds in folk medicine (Ardekani et al. 2010). Variations in factors such as date variety growing conditions, maturity, season, geographic origin, fertiliser, soil type, amount of sunlight received and storage conditions might be responsible for the observed differences (Shahidi et al. 1997).

concentration was more remarkable for olive oil up to 21 days. After 14 days of assay, all of seed extracts was effective at 60 °C in comparison with control. From these results, date seed extract showed significant antioxidant activities in olive oil. The degrees of influence of the extracts were different and it could be change because of the chemical structure and olive oil's own antioxidant capacity. So further studies are necessary to establish a correlation between the antioxidant capacity and chemical composition of date seed extracts.

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Conclusion

All extracts exhibited antioxidant activity compared to BHA up to 21 days. When antioxidant effect of extract concentrations were compared with BHA, the effect of 0.5 % extract

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