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Effect of aqueous and methanol extracts of date palm fruit on stability of sun flower oil

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ABSTRACT

Nowadays, finding new resources of vegetal antioxidants in order to use them in food (as an additive or alternative with artificial antioxidants) is an important research subject in the field of food science and technology. In this research methanol and aqueous extracts of two varieties of date palm fruit (Mozafati and Rabi) were obtained. Then, two concentrations of each extract (2000 and 3000 ppm) were applied in free antioxidant-purified sun flower oil. The oils were kept in 68°C for a period of one month. After storage, Peroxide index of oil samples were determined. According to the results, Two extracts had significant difference in case of total phenolic content ($p < 0.05$) and methanol extracts of Rabi variety had higher that Mozafati in these case. Oil samples containing 2000 ppm methanol extracts of Rabi, had better quality and stability than other samples and had no significant difference with BHT at 200 ppm concentration.

Key words: date palm fruit, Total phenolic content, sun flower, oil stability.

INTRODUCTION

Oxidation of oils is one of the major changes that take place during the processing, distribution, and final preparation of foodstuff. These events not only bring about the loss of the nutritional quality of food but also release oxidized products such as free radicals that result in reduced shelf – life of oils and cause heart disease, cancer and early aging in consumers of these oils. Therefore, stabilizing oils against oxidation is necessary (Yen et al. 1997; Yen and Hsieh 1998).

It has been reported that the oxidation of lipids is substantially reduced by adding antioxidants to oils and fats (Hertog et al. 1993). Synthetic antioxidants are effective as oil preservatives, but their application has been limited in many countries because adverse side effects of their use have been reported. Although synthetic antioxidants are used at low concentrations, there is a need for having antioxidants without side effects because the complications resulting from the long term use of these compounds in man cannot be ignored. Therefore, the search for substitutes of synthetic antioxidants has led to the study of numerous antioxidants found in plants.

The fruit of the date palm (Pheonix dactylifera) is an important commercial crop in the Middle Eastern countries. Date fruits are still considered by many people in this part of the world as a staple food (Sawaya et al. 1982). Date palm is a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium and a significant amount of calcium (Anwar-Shinwary 1987). Besides nutritional value, date fruits are rich in phenolic compounds that have in vitro antioxidant and antimutagenic properties (Vayalil 2005; Osman et al. 2012; Shahdadi

et al. 2013). Purpose of this research was to measure the total content of phenolic compounds methanol and aqueous of date palm fruit and to investigate the effects of these extracts on the stability of sun flower oil.

MATERIALS AND METHODS

Two varieties of date were used in this study, Rabi date and Mozafati date that are grown mostly in Iran shahr city of Iran. The samples were obtained from Iran shahr distribution centre. All chemical compounds (Folin–Ciocalteu, etc.) were bought from the German company Merck, and the synthetic oxidant from the American company Sigma.

Extraction of antioxidants from date fruit

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 domesticated blender (Panasonic, Penang, Malaysia). The extraction solvents were 300 ml methanol–water (4:1 v/v), and 300 ml water. Extraction carried out at ambient temperature (20 °C) for 24 h using a laboratory shaker. The ratio of methanol and water which lead to the highest yield of phenolic compounds and flavonoids during preliminary trials selected as best ratio. Similar ratio of methanol to water was used by biglari et al. (2008). Each extract was filtered with whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laborator). Then all the extracts were dried by a freeze dryer and dried sample constituents stored at 4 °C until use (Arabshahi-Delouee and Urooj 2007).

Estimation of total phenolic compounds

Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard, and Singleton 1977). Briefly, 20 µl of extract solution were mixed with 300 µl of Na2CO3 solution (20 %), then 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents by using the following linear equation were obtained from calibration curve:

$$A=18571C-13.33$$
$$R^2=0.995$$

Where A is the absorbance and C is concentration as gallic acid equivalents (mg/ml).

Preparing sun flower oil samples

Methanol and aqueous extracts of date fruit at two concentrations (2000 and 3000 ppm) and the synthetic antioxidant BHT at the concentration of 100 and 200 ppm were added to antioxidant free sun flower oil in clean glass containers and thoroughly mixed for a few minutes for the antioxidants to be completely dispersed in oil. The oil samples were then kept at 68°C for 4 weeks and their peroxide value were measured on the same day, and at Once a week.

Statistical analysis

All these experiments were replicated three times (from 3 different batches of samples), and the average values are reported. Analysis of the variance of the data was performed using the completely randomized design. Comparison of the means of the data was carried out using the SAS software (2008) based on Duncan's multiple range tests at the 5 percent probability level.

RESULTS AND DISCUSSION

Total phenolic compound (TPC)

Due to variety and growth conditions, DPF vary in shape, size, weight and moisture content and this may affect phenolic compounds. The averages of total phenolic compound of date palm fruit based on Folin-Ciocalteau method were shown in table 1.

Table 1 Effect of solvent and variety on total phenolic compound Date Palm Fruits.

Means with same superscripts had no significant difference with each other (P >0.05)

Solvent	Rabi variety	Mozafati variety
methanol	3.35^{a}	1.77^{b}
Water	1.15^{b}	0.5^{c}

As can be seen from table 1 date varieties have significant differences (P <0.05) in total phenolic content. Among studied varieties, Rabi contained the higher amount of total phenolic in comparison with Mozafati. These results showed that date palm fruit grown in Iran shahr had a similar level of phenolic content with those of Kerman date palm fruit (Shahdadi, et al. 2013) and Oman date palm fruit and also with those of Bahrain dates (Allait, 2008). However, Mansouri et al. (2005) and Biglari et al. (2008) reported that total phenolic content of Algerian and Iranian date palm fruit ranged from 2.49 to 8.36 mg GAE/100 g of fresh weight and from 2.89 to 6.64 mg GAE/100 g of dry weight respectively. Kharak date (Iranian dry date) showed an average of 141.35 mg GAE/100 g dry weight. In the other way, the study reported by Wu et al. (2004), on lipophilic and hydrophilic antioxidant capacities of common foods in the United States found that Deglet Noor and Medjool varieties presented a high level on total phenolic content (661 and 572 mg of GAE per 100 g fresh weight respectively) as compared to our study. Various factors such as variety, growing condition, maturity, season, geographic origin between the two countries, fertilizers, soil type, amount of sunlight received and experimental conditions (storage, extraction solvent) among others might be responsible for the observed differences.

The selection of extraction solvents is critical for the complex food samples as it will determine the amount and type of phenolic compounds being extracted. Aqueous alcohols particularly acetone, ethanol and methanol are most commonly employed in phenolic compound extraction from botanical materials (Naczk and Shahidi 2004; Hayouni et al. 2007). Table 1 showed that methanol extract of Rabi variety had the highest extraction level of phenolic compounds (3.35 mg GAE/ml)

Because polyphenols are mostly polar compounds, highly-polar solvents (e.g. water) and non-polar ones (e.g. chloroform and hexane) are not appropriate for extracting a high phenolic content. Moreover, the use of water as the only solvent yields an extract with a high content of impurities (e.g. organic acids, sugars, soluble proteins) along with phenolic compounds which could interfere in the phenolic identification and quantification. On the other hand, the absolute alcoholic solvents decrease extraction yield. So, application of water combined with other organic solvents makes it a moderately polar medium ensuring the optimal conditions for extraction of polyphenols. Besides, using water in combination with alcohols leads to an increase in swelling of plant materials and the contact surface area between the plant matrix and the solvent which finally, improves the extraction yield (Chirinos et al. 2007).

Many studies have confirmed that also in other plant species polar solvents produce a higher yield of phenolic concentration compared with the non-polar ones (Trabelsi et al. 2008; Franco et al. 2008). Furthermore, the higher level of phenolic content in alcoholic extracts than that in water extracts of *Hieracium pilosella* was proved in the study conducted by Stanojević et al (2009).

Investigate the antioxidant extract of date fruit on sunflower oil:
Average peroxide of various treatments of sunflower oil during 4 weeks of storage at 68 °C is shown in Table 2.

Table 2. Means of peroxide value (meq/kg) of oil samples kept for 4 weeks at 67°C

Duration of keeping the oil (weeks)	0	1	2	3	4
Sun flower oil (SF)	15.4^{m}	57.2^{j}	84.36^{h}	120.35^{e}	169^{a}
SF + 100 ppm BHT	11.4^{m}	45.3^{k}	68.8^{i}	120^{e}	158.3^{b}
SF + 200 ppm BHT	3.6^{n}	9.3^{m}	27^{l}	59.8^{j}	90.5^{g}
SF + 2000 ppm Methanol, Rabi	3.4^{n}	9.7^{m}	24.4^{l}	61.1^{j}	87.4^{g}
SF + 3000 ppm Methanol, Rabi	10.2^{m}	23.34^{l}	59.8^{j}	83.4^{h}	129.4^{d}
SF + 2000 ppm water, Rabi	12.4^{m}	20.57^{l}	67.8^{ij}	89.53^{g}	165.3^{a}
SF + 3000 ppm water , Rabi	11.9^{m}	20.2^{l}	61.34^{j}	80.3^{h}	144.1^{c}
SF + 2000 ppm Methanol, Mozafati	13.5^{m}	43.5^{k}	76.8^{hi}	103.1^{g}	134.6^{d}
SF + 3000 ppm Methanol, Mozafati	11.3^{m}	39.4^{k}	71.5^{i}	90.5^{g}	112.4^{f}
SF + 2000 ppm water, Mozafati	12.5^{m}	43.3^{k}	84.2^{h}	162.54^{a}	139.7^{d}
SF + 3000 ppm water, Mozafati	10^{m}	39.1^{k}	59.2^{j}	103.3^{g}	130.2^{d}

As can be seen in table 2, sun flower oil samples always exhibited greater resistance to a rise in peroxide value when higher concentrations of extracts were added to them except at methanol extracts of Rabi. The reason for this is the presence of phenolic compounds, and other antioxidants, in these extracts: when higher concentrations of extracts were added to sun flower oil, they were more effective in inhibiting a rise in peroxide value. In the different treatments of sun flower oil, the oil sample containing 100 ppm BHT and 2000 ppm Rabi methanol extracts had the

lowest peroxide value, while the oil samples which contained 3000 ppm Rabi Methanol extract and 3000 ppm Mozafati aqueous extract had the second and the third lowest peroxide value, respectively.

In the beginning, extracts (at 2000 and 3000 ppm) or BHT (at 100 and 200 ppm) were added to sun flower oil, no significant differences could be observed in peroxide value except oil samples with 200 ppm BHT and 2000 ppm Rabi Methanol extract.

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All analyzed samples for peroxide value had significant difference with the control sample. Antioxidants remain active in a specific period, and over time their impact gradually reduced that this can be due to samples storage at high temperature oxidation conditions. Therefore, by increasing the storage time of the oil samples, peroxide number increased.

There are no reports on the use of date palm fruit extracts on oils stability in literatures but a lot of research on the effect of plant extracts on the oil stability.

Results of the study conducted by Kamali Roosta et al. (2011) on the effects of adding different concentrations (0.02, 0.04, 0.06, 0.08, and 0.1 percent) of methanol and acetone extracts of cinnamon to purified sunflower oil, and of keeping the oil at 90°C for 120 h, showed that the trend of inhibiting an increase in the peroxide value gained strength when higher concentrations of the extracts were added to the oil.

In their study, Abdelaal and Halaweish (2010) found that the ambient temperature and the length of time passed after the addition of the extracts to soybean oil affected the development of oxidative rancidity of the oil. 7 days after the oil to which the extract had been added was kept at 65°C, significant increases were observed in the peroxide value of the oil samples.

Mirahmadi et al. (2006) observed that the antioxidant effect of the extract of green tea leaves at concentrations of 200 and 500 ppm in preventing the oxidation of sunflower oil were greater than those of the synthetic antioxidants BHA and BHT (at 100 and 200 ppm), and that they were also greater than that of tocopherol (at 200 and 500 ppm).
Rafiei et al. (2011) investigated the antioxidant activities of extracts of olive leaves at concentrations of 200, 500, and 1000 ppm on sunflower oil and compared these effects with those of the synthetic antioxidants BHA and BHT at concentrations of 100 and 200 ppm. They reported that the effects of treatments and those of the lengths of time the antioxidants were added to the oil were significant at the 5 percent probability level, that the higher concentrations of the methanol extract were more successful in the surveillance of the peroxide value, and that the methanol extract at 1000 ppm could replace the use of both concentrations of the synthetic antioxidants.

Rezai et al. (2012) studied the antioxidant activity of the methanol extract of walnut leave on soybean oil, and reported that these extracts at 1000 ppm were more effective in reducing the peroxide value of the oils than the synthetic antioxidant BHA and BHT at 200 ppm.

CONCLUSION

According to the oxidative stability index in Table 2, the 3000 ppm extracts date palm fruit were more effective than the 2000 ppm extract in prolonging the stability of sunflower oil except in Rabi methanol extract. This could be attributed to the higher concentrations of phenolic compounds in these extracts. The effect of date palm fruit extracts in stabilizing unstable oils like sunflower oil was comparable to that of the common synthetic antioxidant. This finding, which is a significant one, was confirmed by the results obtained concerning the factor of the peroxide value. Therefore, it can be concluded that extracts of date fruit can be used as natural and safe antioxidants in oils, fats, and other foodstuff to prevent and stop their oxidation.

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