International Food Research Journal 19(1): 223-227 (2012)

Antioxidant activities of date pits in a model meat system

[1]Amany, M. M. B., [2]Shaker, M. A. and [3]Abeer, A. K.

[1]Food Science and Nutrition Department, Faculty Agriculture Science and Foods, King Faisal University, Saudi Arabia
[2]Department of Fats and Oils, Food Technology Research Institute, Agriculture Research Centre, Giza, Egypt
[3]Department of Consumer Science, Faculty Agriculture Science and Foods, King Faisal University, Saudi Arabia

Abstract: The aim of the current investigation was to investigate the effect of date pits phenolic compounds compared to BHT as synthetic antioxidant on lipid oxidation and quality of ground beef during refrigerated storage at 0.00 ± 0.50˚C for up to 10 days. Khalas variety of date pits (*Phoenix dactylifera* L.) phenolic compounds was extracted with 4 solvents (Water, methanol, methanol: water (50:50 v/v) and methanol: water: acetone: formic acid (20:40:40:1). Ferric reducing antioxidant power assay (FRAP) and Folin-Ciocalteu reagent was used for determination of the antioxidant activity and phenolic content of date pits. Results indicated that the highest antioxidant was shown by the date pits extract (Water: methanol: acetone: formic acid), therefore 0.5, 0.75 and 1.00 % of either date pits extract and BHT were added to minced meat and evaluate it's effects on the lipid peroxidation of ground beef during storage process. TBA test as quality assurance test was conducted at the beginning of the experiment and after 2, 4, 6, 8 and 10 days of storage. The results of this study showed that the date pits extract (Water: methanol: acetone: formic acid) had significantly the highest levels of total polyphenols and antioxidant activity. Also, the obtained results indicated that phenolic compounds in date pits of khalas variety had high antioxidative effect in reducing the formation of hydroperoxides during storage.

Keywords: Phenolic compounds, antoxidative activity, date pits, FRAP method, fatty food

Introduction

Dates of date palm tree (*Phoenix dactylifera* L.) are popular among the population of the Middle Eastern Countries. The fruit is composed of a fleshy pericarp and seed which constitutes between 10% and 15% of date fruit weight (Hussein *et al*., 1998). The date seeds considered a waste product of many date processing plants producing pitted dates, date syrup and date confectionery. At present, seeds are used mainly for animal feeds in the cattle, sheep, and camel and poultry industries. With world production of dates reaching 9 million tones in 2007, from this approximately 960 thousand tones of date seeds are produced (FAO, 2010). Thus, utilization of such waste is very important to date cultivation and to increase the income to this sector (Al-Farsi *et al*., 2008). Chemical and nutritional constituents of date seeds were reported by (El-Rayes, 2009 and Mobarak 2009; Basuny and Al-Marzooq, 2010; Ardekani *et al*., 2010). Most current literature was limited to proximate and mineral composition. Beyond compositional analysis, there is work of (Al-Farsi and Lee 2007) who researched the functional properties of date seeds. Their reported composition was 3.10-7.10% moisture, 2.30-6.40% protein, 5.00-13.20 fat, 0.90-1.80% ash and 22.50-80.20% dietary fiber. Also seeds contain high levels of phenolics (3102-4430 mg Gallic acid 1100 gm (Ardekani *et al*., 2010).

Antioxidant compounds have been identified in grape seeds (Shi *et al*., 2003 & Basuny, 2004), olive leaf (Farag *et al*., 2007), pomegranate peels (Allam and Basuny 2002). Phenolic compounds of fruit seeds mainly phenolic acids and flavonids, have been shown to possess such as antioxidant, anticarcinogenic, antimicrobial, antimutagenic and antiinflamatory activities (Halliwell, 1997; Diplock *et al*., 1998), as well as reduction of cardiovascular diseases (Peterson and Dwyer, 1998). Thus it is considered important to increase the antioxidant intake in the human diet and one way achieving this is by enriching food with phenolics. The synthetic antioxidants, i.e: butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are very cost-effective given a high stability. The use of BHT and BHA in food has been decreased due to their suspected action as promoters of carcinogenesis, as well for the general consumer rejection of synthetic food additives (Namiki, 1990). Several studies indicated that the use of synthetic antioxidants has begun to be restricted because of their health risks and toxicity (Farag *et al*., 2006). In this respect Tariq *et al*. (2000) reported the pathological changes such as wide extension at hepatocellular degeneration including cloudly swelling and vacuolar degeneration, mostly fatty degeneration produced in rats after 6-weeks administration of BHT at levels

*Corresponding author.
Email: *dramany_basuny@yahoo.com*

200 ppm. Therefore, the importance of replacing synthetic antioxidants with natural ingredients from oilseeds, spices and other plant materials has greatly increased. Some components isolated from fruits and vegetable has been proven in model systems, being effective as antioxidants as synthetic antioxidants (Basuny, 2004; Farag *et al*., 2007).

Accordingly, in the current study Phenolic compounds from date pits were extracted with 4 solvents and evaluated as natural antioxidant by ferric reducing antioxidant power (RARP). Phenolic compounds present in date pits were added to ground beef at various levels (0.5, 0.75 and 1%) compared with synthetic antioxidant (BHT) on the stability and quality of ground beef by determination the changes in TBA value during refrigerated storage and to evaluate the effect adding different levels of date pits extract on sensory properties of cooked ground beef at the end of storage period. The main goal of this work was to use very cheap natural source to act as antioxidant agent.

Material and Methods

Source of pits

The pits of khalas variety was directly isolated from 50 kg of waste date fruit having the Al-Hasa region, Saudi Arabia and collected at the tamr stage (fruit ripeness).

Solvents and standard reagents

All solvents used throughout the whole work were of analytical grade and distilled before use. BHT was purchased from Sigma (st. Louis. MO. USA). Folin-Cioculteau reagent was obtained from Gerbsaur Chemical Co (Germany). Gallic acid (98%) was purchased from Aldrich Chemical Co. Ltd., England.

Pits extraction

Pits of khalas variety were separately milled in a heavy-duty grinder to pass 1-2 mm screens and then preserved at 2-8˚C until analysis. About 0.02 gm powdered date pits was shacked with 5 ml of solvent in a glass tube at room temperature, two times for 30 min and centrifuged. Water 5 ml methanol: water (50:50, v/v), methanol, water: methanol: acetone: formic acid (20:40:40:0.1) were used as the best solvents. The extraction was carried out using four different solvents to compare the antioxidant activities and the total phenolic contents of each extract.

Measurement of the total phenolic content

Total phenolics were determined calorimetrically using Folin-Ciocateau reagent as described by Velioglu *et a*l. (1998) with slight modifications. The prepared extract (200 µl) was mixed with 1.50 ml of Folin-Ciocateau reagent (previously diluted 10 fold with distilled water) and allowed to stand at 200˚C for 5 min. A 1.50 ml sodium bicarbonate solution (60 g/l) was added to the mixture. After 90 min at 22˚C, the absorbance was measured at 725 nm using a UV spectrophotometer. The total phenolics were quantified by the calibration curve obtained from measuring the absorbance of a known concentration of Gallic acid standard 20-150 mg/l). The concentrations were expressed as equivalent milligrams of Gallic acid per 100 g dry pits.

Evaluation of antioxidant activity using FRAP method

The FRAP (ferric reducing antioxidant power assay) procedure described by Benzie and Steain (1996). The principle of this method is the reduction of a ferric-tripyridyl triazine complex to its colored ferrous from in the presence of antioxidants. Briefly, the FRAP reagent contained 5 ml of 10 mmol/L solution of TPTZ (2,4,6-tripyridyl-5-triazine) in 40 mmol/L HCL plus 5 ml of a 20 mmol/L solution of $FeCl_3$ and 50 ml of a 0.3 mol/L acetate buffer solution, pH 3.60 which was prepared freshly and warmed at 37˚C. Aliquots of 50 µl extract were mixed with 1.50 ml FRAP reagent and after incubation at 37˚C for 10 min, the absorbance of the reaction mixture was measured at 593 nm. For constructions of the calibration curve, five concentrations of $FeSo_4$, $7H_2O$ (1000, 750, 500, 250, and 125 µl/L) were used and the absorbance values were measured as for sample solutions. The antioxidant activities were expressed as the concentrations of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSo_4$.

Preparation of ground beef

Beef meat (5 kg) was cut into small pieces and homogenized in stainless steel blender. Ground beef were mixed by latex gloved hands with 0.50, 0.75, and 1.00% of either the highest antioxidant efficiency of date pits extracts and BHT. Minced beef without additives was run as control. The abovementioned samples were packed in polyethylene bags, each bag contain 250 g and stored at 0 ± 0.5˚C in refrigerator for 10 days.

Thiobarbituric acid-reactive substances (TBARS)

The TBARS values were determined in triplicate samples by the extraction method of Mielnik *et al*. (2006). For extraction 10g meat was homogenized with

30 ml of a 7.50 % aqueous solution of trichloroacetic acid (TCA). After filtration, 5.00 L^{-1} of 0.02 L^{-1} aqueous thiobarbituric acid (TBA) in a stoppered test tube. The samples were incubated at 100˚C for 35 min in cold water-bath and subsequently cooled for 10 min in cold water. Absorbance was measured at 532 nm by using UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). Against a blank containing 5 ml distilled water and 5 ml TBA reagent. Results expressed as milligrams malondialdehyde kg^{-1} meat.

Cooking and sensory evaluation

Minced beef samples were mixed with 1% salt into a mould and cooked for 30 min in water bath at 75˚C until an internal temperature of 75˚C was reached. Cooked beef samples were left to be warm then served for sensory evaluation. Sensory evaluation method was conducted according to the method described by Mansour and Khalil (2000), cooked beef samples were served warm to 10 members trained panel (Staff of Food science and nutrition, depart. Faculty of agricultural Science and Foods, King Faisal University, Al-Hasa, Saudi Arabia) without care of age or sex. The panelists were subjected to sensory evaluation using a 9-point hedonic scale for taste, color, odor and overall acceptability. A numerical basis as a sort of evaluation from 1 to 9 was used where (1 =dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8, like very much, 9= like extremely.

Statistical analysis

The data of the present study were subjected to analysis of variance and the least significant difference test, in order to compare the mean values of the investigated parameters.

Results and Discussion

To compare the effects of the extraction methods on antioxidant activities and total phenolics contents in khalas variety of date pit, four different solvents were used (Figures 1 and 2). All the extracts had considerable antioxidant activities from the 9.77 mmol of Fe^{++} /100 g dry plant in pits of khalas variety (methanol) to 38.00 mmol of Fe^{++} /100 g formic acid. All the extracts contained considerable phenolic contents from the equivalent 900 µg Gallic acid /g dry plant in pits (methanol) to 2015 µg Gallic acid /g dry plant in pits (formic acid). Significant differences ($P \geq 0.05$) existed among different solvents used, with some exception. Extraction by the formic acid gave the highest antioxidant activity and total phenolic content, whereas methanol resulted in the lowest values. The results showed that most of the potent antioxidant and phenolic content in the pits of khalas variety were soluble in formic acid. One could report that there is high relationship between the content of total polyphenols of extract and its antioxidant activity; hence the content of total polphenol of extract is increased as antioxidant activity of extract increased. The high antioxidant activity may be due the high concentration of phenolics in data pit extracts. These compounds are an important group of natural antioxidant with possible beneficial effects on human health (Meyers *et al*., 2003). They can participate in protection against the harmful action of reactive oxygen species, mainly oxygen free radicals. These compounds are known also to passes antioxidant activity due to its stability to reduce free radical stability via electron or hydrogen-donating (Lee *et al*., 1999). The antioxidants activity of polyphenols is principally based on the properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure (Rice-Evan *et al*., 1996).

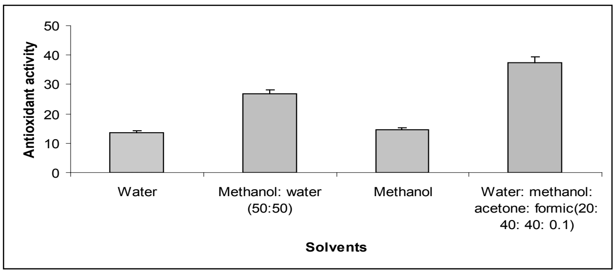

Figure 1. Antioxidant activity of Khalas variety of date pits using 4 different solvents (as mmol Fe^{++}/ 100 g). Data are expressed as mean ± SE. Each sample was analyzed three times.

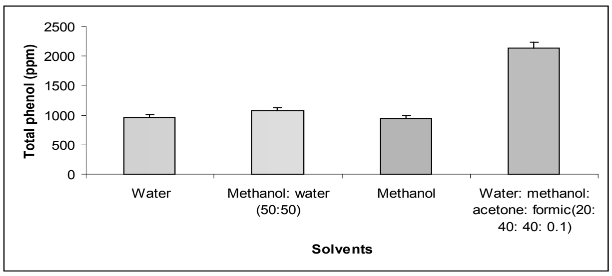

Figure 2. Total phenol contents of Khalas variety of date pits using 4 different solvents (as µg Gallic acid / g). Data are expressed as mean ± SE. Each sample was analyzed three times.

Chemical composition of ground beef

The moisture content of ground beef was 60.00%. The protein, fat, ash contents of ground beef were 50.10, 38.00 and 4.00%, respectively. The obtained results indicate that beef contain relatively high levels of fat which consider a major cause of quality deterioration in meat and its cooked products (Figure 3).

Changes of TBA value of ground beef during refrigerated storage

Ground meat tends to become rancid and brown more rapidly, due to pigment and lipid oxidation. An oxidative reaction in muscle foods leads to degradation of lipid and proteins, resulting in deterioration of flavour, texture and nutritive value and is considered as one of the major problems in the development of new convenient meat products and processes (Gray and Pearson, 1987). In the present investigation based on the antioxidant activities results, the highest antioxidant activity was shown by the formic acid, there fore 0.5, 0.75 and 1.00 % of either date pits extract and BHT were added to minced meat to evaluate its effects on the lipid peroxidation of ground beef during storage process. Generally, TBA values increased gradually and significantly ($P \geq 0.05$) during storage period. The phospholipids in muscle membrane provide an ideal substrate for lipid peroxidation. Iron bound to negatively charged phospholipids promotes lipid peroxidation, resulting in generation of warmed-over flavor (Empson *et al*., 1991). However, mixing minced beef with various levels of khalas date pits caused a significant ($P \geq 0.05$) reduction of TBA values compared to control sample. These inhibitory effects of date pits were dose-dependent. Control samples had significantly the highest TBA value was 2.00 mg malondialdehyde kg^{-1} at the end of the storage period, the highest TBA values of the control sample might be due to an interaction between the natural substances (for example, polyunsaturated fatty acids) and catalysts (for example, iron ion) from the meat tissue during storage (Kim *et al*., 2000), while beef samples mixed with 1.00% date pits extract had significantly ($P \geq 0.05$) the lowest TBA values was 0.69 mg malondialdehyde kg^{-1} at the end of the storage period. No significant differences were observed in TBA values of ground beef mixed with 0.75% date pits extract and that samples treated with 1.00% of synthetic antioxidant BHT. The obtained results indicate high antioxidative effect in reducing the formation of hydroperoxides during storage process (Figure 3). These results are in good agreement with those obtained by (El-Rayes, 2009). In this respect the results of Abd El-Hamied *et al*. (2009) observed that the addition of rosemary, sage and their combination showed high antioxidative effects during refrigerated and frozen storage of minced meat. Also Basuny (2004) show that mixing oil with various levels of grape seed phenolic compounds caused significant decrease of the formation of secondary products during frying process.

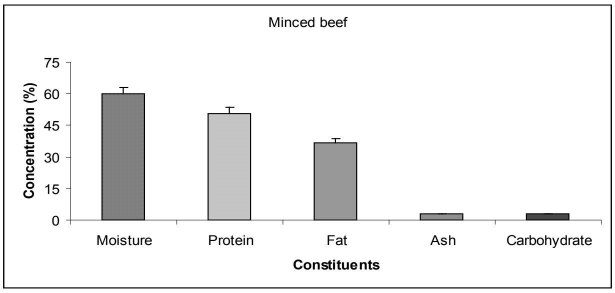

Figure 3. Chemical composition of minced beef (based on dry weight basis). Data are expressed as mean ± SE. Each sample was analyzed three times.

Sensory evaluation

Sensory characteristics of cooked minced beef as affected by adding different levels of date pits extract and BHT are presented in Figure 4. Treated samples had relatively high value of taste, odor, color and overall acceptability compared to control sample. No significant differences were observed in taste between samples mixed with 0.5 and 0.75% of date pits extract to those mixed with 0.5% of BHT. All samples showed good overall acceptability; in all cases the values were higher than 6.00. However samples mixed with 0.75 and 1.00 of date pits extract had significantly ($P \geq 0.05$) the highest score of overall acceptability were 8.60 and 8.50, respectively. The results of sensory evaluation suggest that the date pits phenolic compounds can be successfully used as natural antioxidant.

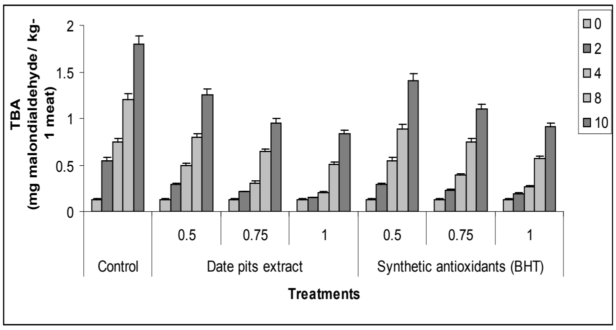

Figure 4. Effect of date pits extract and BHT on TBA number (mg malondialdehyde kg^{-1} meat) of minced beef during storage at 0˚C. Data are expressed as mean ± SE. Each sample was analyzed three times.

Conclusion

The obtained results showed that the antioxidant activity of date pits phenolic compounds and used in fatty food, cosmetics, and pharmaceuticals.

References

Abd-Ei-Hamied, A. A., Nassar, A. G. and El-Badry, N. 2009. Investigation on antioxidant and antibacterial activities of some natural extracts. World Journal Dairy Food Science 4: 1-7.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M. and Al-Rawahy, F. 2008. Compositional and functiona characteristics of dates, syrups and their by-products. Food Chemistry 104: 943-947.

Al-Farsi, A. M. and Lee, C. Y. 2007. Optimization of phenolics and dietary fiber extraction from date seeds. Food Chemistry 108: 977-985.

Allam, S. M. and Basuny, A. M. 2002. Chlorogenic acid, efficiency and safety aspects as antioxidant. La Rivista Italiana Delle Sostanze Grasses LXXIX: 257-265.

Ardekani, M. R., Khanavi, M., Hajimahmoodi, M., Jahangiri, M. and Hadjiakhoondi, A. 2010. Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. Iranian Journal of Pharmaceutical Research 9: 141-146.

A. O. A. C. 2000. Official Methods of Analysis of the Association of Analytical, 18th Edn., AOAC, Washington DC, USA.

Basuny, A. M. 2004. Influence of grape seed phenolic compounds on thermal stability of frying oils. Egypt. Journal Food Science 32: 65-78.

Basuny, A. M. and Al-Marzooq, A. M. 2010. Production of mayonnaise from date pits oil. Banat's Journal of Biotechnology 2: 3-8.

Benzie, I. F. F. and Steain, J. J. 1996. The reducing ability of plasma as a measure of antioxidant power: FRAP assay. Analytical Biochemistry 239: 70-76.

Diplock, A., Charleux, J., Wilii, G., Koko, K., Rice-Evans, C. and Roberfroid, M. 1998. Functional food sciences and defense against reactive oxidative species. British Journal of Nutrition 80: 77-82.

El-Rayes, D. A. 2009. Characterization of three date palm cultivars based on RAPD finger prints and fruit chemical composition. Environmental and Arid Land Agriculture Science 20: 3-20.

Empson, K. L.; Labuza, T. P. and Graf, E. 1991. Phytic acid as a food antioxidant. Journal Food Science 56: 560-563.

FAO. 2010. Statistical databases. *http://faostat.fao.org* Accessed June 2010.

Farag, R. S., Mohamoud, E. A., Basuny, A. M. and Ali, R. 2006. Influence of crude olive leaf on rat liver and kidney function. International Journal of Food Technology 41: 1-10.

Farag, R. S., Ebtesam, A. M. and Basuny, A. M. 2007. Use crude olive leaf juice as a natural antioxidant for the stability of sunflower oil during heating. International Journal of Food Science and Technology 42: 107-115.

Gray, J. I. and Pearson, A. M. 1987. Rancidity and warmed-over flavor. Advances Meat Research 3: 221-269.

Halliwell, B. 1997. Antioxidants and human disease: A general introduction. Nutrition Reviews, 55: 44-52.

Hussein, A. S., Alhadrami, G. A. and Khalil, Y. H. 1998. The use of dates and dates pits in broiler starter and finisher diets. Bioresource Technology 66: 219-223.

Kim, J. S., Godber, J. S. and Prinaywiwatkul, W. 2000. Restructured beef roasts containing rice bran oil an fiber influences cholesterol oxidation and nutritional profile. Journal Muscle Food 11: 11-137.

Lee, S. E., Lee, H. S. and Ahn, Y. 1999. Scavenging effect of plant derived materials on free radicals and active oxygen species. Agricultural Chemistry & Biotechnology 42: 40-44.

Mansour, E. H. and Khalil, A. H. 2000. Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties, Food Chemistry 69: 135-141.

Meyers, K. J., Watkins, C. B., Pritts, M. P. and Liu, R. H. 2003. Antioxidant and antiproliferative activities of strawberries. Journal Agricultural Food Chemistry 51: 6887-6892.

Mielnik, M. B., Olsen, G., Vogt, D., Adeline, E. and Skrede, G. 2006. Grape seed extract as antioxidant in cooked, cold stored turkey meat. LWT 39: 191-198.

Mobarak, S. A. 2009. Antioxidant properties of date palm (*Phoenix dactylifera* L.) cultivars. Ph.D. Colorado State University.

Namiki, M. 1990. Antioxidants / Antimutagenes in foods. Critical Review in Food Science and Nutrition 29: 273-279.

Peterson, J. and Dwyer, J. 1998. Flavonoids: Dietary occurrence and biochemical activity. Nutrition Research 18: 1995-2018.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. 1996. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radicals and Biological Medicine 20: 933-956.

Shi, J., Yu, J., Pohorly, J., Young, C., Bryan, M. and Wu, Y. 2003. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. Food Agriculture and Environment 1: 42-47.

Tariq, N., Jenkins, D. J. A., Vidgen, E., Fleshner, N., Kenndall, C. W. C. and Story, J. A. 2000. Effect of soluble and insoluble fiber diets on serum prostate specific antigen in men. The Journal of Urology 163: 114-118.

Velioglu, Y. S., Mazza, G., Goo, L. and Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetable and grain products. Journal Agriculture Food Chemistry 46: 4113-4117.