J.Basrah Researches (Sciences) Vol.32. Part.2. 82-87 (2006)

Antioxidant acitivity of dried orange

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ISSN –1817 –2695
Received 21/12/2005, Accepted 28/5/2006

Abstract

The antioxidant properties (antioxidant activity, chelating activity to ferric ion and total phenolic compounds) of ground dried orange were studied. Antioxidant activity was studied in both system. The oxidation of linoleic acid compaired with synthetic antioxidant. Butylated hydroxy toluene (BHT) system and the ground beef system. At three levels 2%, 4% and 6% and the sample (raw and cooked) stored in refrigerator at $5C^o$ for 9 days another samples stored at freezing ($-18 \pm 2C^o$) for 6 months. The results showed that the ground dried orange has antioxidant activity compaired with BHT, and it exhibited ferric ion chelating capability. The thiobarbituric acid test indicated that at 2%, 4% and 6% (w/w) levels dried orange reduced rancidity development in both frozen and unfrozen meats. There were non significant differences among various concentration of dried orange on the thiobarbituric acid value.

Introduction

An antioxidant can be defined as any substance that when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate. (Atoui, *et al.*,2005). The formation of potentially toxic compounds caused by the oxidative deterioration of lipids in foods, is responsible for the decrease in food quality and safety (Moure *et al.*,2001), it is necessary to suppress lipid peroxidation in food inorder to preserve flavor, color and nutritional value. The addition of antioxidants to food is the most effective way for delaying lipid peroxidation which is the reason for the unpleasant flavors (Frankel,1996).In food industry, synthetic antioxidants, such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and propyl gallate (PG), are widely used because they are effective and less expensive than natural antioxidants. Their safety issues, however, are highly debated, thus generating the need to search for substitute materials from natural and safe source as food antioxidants. References much attention has been focused on vitamins C, E and carotenoids (Mallet, 1994; Tsuchihashi *et al*.,1995).Orange is the first and most important citrus fruit in both fresh consumption and industrial uses. It is one of the main crops in Iraq. In orange several compounds have antioxidant activity such as flavonoids, malic, citric and ascorbic acids (Nagy,1980; Bocco *et al.*,1998). Various polyphenolic acids and their derivatives which also occur in oranges have been shown to have antioxidant activity (Kefford and chandler,1970; Ubando-Rivera *et al.*,2005). The purpose of this study is to investigate the antioxidant activity of dried orange which retard rancidity in the products to which it was added.

Material and Methods

Sample preparation

Three kilograms of orange, *Citrus Sinesis* were juiced coarsely by press, the pulp and peel ground and dried under vaccum at $70C^o$ for approximately 15hr. The dried lots were finely ground (mesh 16) and all of the lots were mixed and used in the experiments which lasted approximately 6 months. Meat loaves were prepared by mixining 50gm ground beef and 0.5 gm salt with 2% or 4% or 6% (w/w) dried orange. Some samples were baked

immediately in foil-lined loaf and cooked at 225C^o for 15 min. The other to be tested uncooked were wrapped in foil and both samples (cooked and uncooked) stored in refrigerator at 5C^o and in freezer at (-18 ± 2C^o) for 6 months. During the storage periods the Thiobarbituric acid (TBA) was determined .Statistically significant differences were determined by the analysis of variance using a randomized block design.

Chemical analyses:

1-Determination of antioxidant activity (0.5, 1, 1.5, 2, 2.5, 3) mg of dried orange or BHT was dissolved in 4ml of 95% (w/v) ethanol and mixed with linoleic acid (2.51% in absolute ethanol) (4.1ml),0.5 M phosphate buffer pH 7.0 (8ml), and distilled water (3.9ml), and kept in screw cap containers at 40C^o for 24hr. in the dark. To 0.1ml of this solution was then added 9.7ml of 75% ethanol and 0.1ml of 30% (w/v) ammonium thiocyanate. Precisely 3min after the addition of 0.1ml of 20mμ ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500nm of the resulting red solution was measured. (Hung et al.,2004). The percent inhibition of linoleic acid peroxidation was calculated as:

$$\text{Antioxidative activity (\% inhibition)} = 100 - \left[\frac{\text{absorbance of sample}}{\text{absorbance of control}}\right] \times 100$$

2-Ferric ion chelating effect: Reaction mixtures containing (0.1ml) of dried orange and EDTA was used as positive control of various concentrations (0.2, 0.4, 0.6, 0.8 and 1mg/ml) 0.2ml of 0.5mM ferric sulfate and 0.2ml of 5mM ferrozine were incubated at 37C^o for 10 min. After adding 1.5ml of deionized water to the mixture, the adsorbance at 562nm was measured. The present chelating effect was calculated as:

$$\text{Chelating effect\%} = 100 - \left[\frac{\text{absorbance of sample}}{\text{absorbance of control}}\right] \times 100$$

(Wang *et al*.,2003)

3-Determination of total phenolic compounds.Total soluble phenolics in dried orange were determined with Folin-Ciocalteau reagent according to the method of Slinkard and Singleton (1977), using gallic acid as a standard phenolic compound.1.0ml of extract solution containing 1.0gm of extracts was placed in a volumetric flask and diluted with distilled water (46ml).One millitre of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. After three minutes, 3ml Na_2CO_3 of 2% (w/v) was added, and the mixture was allowed to stand for 2hr with intermittent shaking. The absorbance was measured at 760nm. The concentration of total phenolic compounds in the dried orange was determined as microgram of gallic acid equivalent using an equation obtained from the standard gallic acid graph:

Absorbance = 0.0008 × Gallic acid (μg).

4-The Thiobarbituric acid was determined according to pearson (1970). Macerate 10g fatty food with 50 ml water for 2 min. and wash into a distillation flask with 47.5 ml water. Add 2.5 ml of 4 N hydrochloric acid to bring the pH to 1.5, followed by antifoaming preparation and a few glass beads. Heat the flask by meas of an electric mantle so that 50 ml distillate is collected in 10 min.. from the time boiling commences. Pipette 5 ml distillate into a glass-stoppered tube, add 5 ml TBA reagent (0.2883 g/100 ml of 90% glacial acetic acid), stopper, shake and heat in boiling water for 35 min. Prepare a blank similarly using 5 ml water with 5 ml reagent. Then cool the tubes in water for 10 min. and measure the optical density (D) against the blank at 538 mμ using 1 cm cells.

TBA No. (as mg malonaldehyed per Kg sample) = 7.81)

Results and Discussion

The antioxidant activity of dried orange with the antioxidant activity of Butylated hydroxy toluene (BHT) is shown in Fig. 1.It was noticed that the antioxidant activity increased as the concentration of dried orange was increased.

Figure 2. show that dried orange has good chelating activity to ferric ions which increased oxidation, this chelating activity was increased with the increased concentration of dried orange.

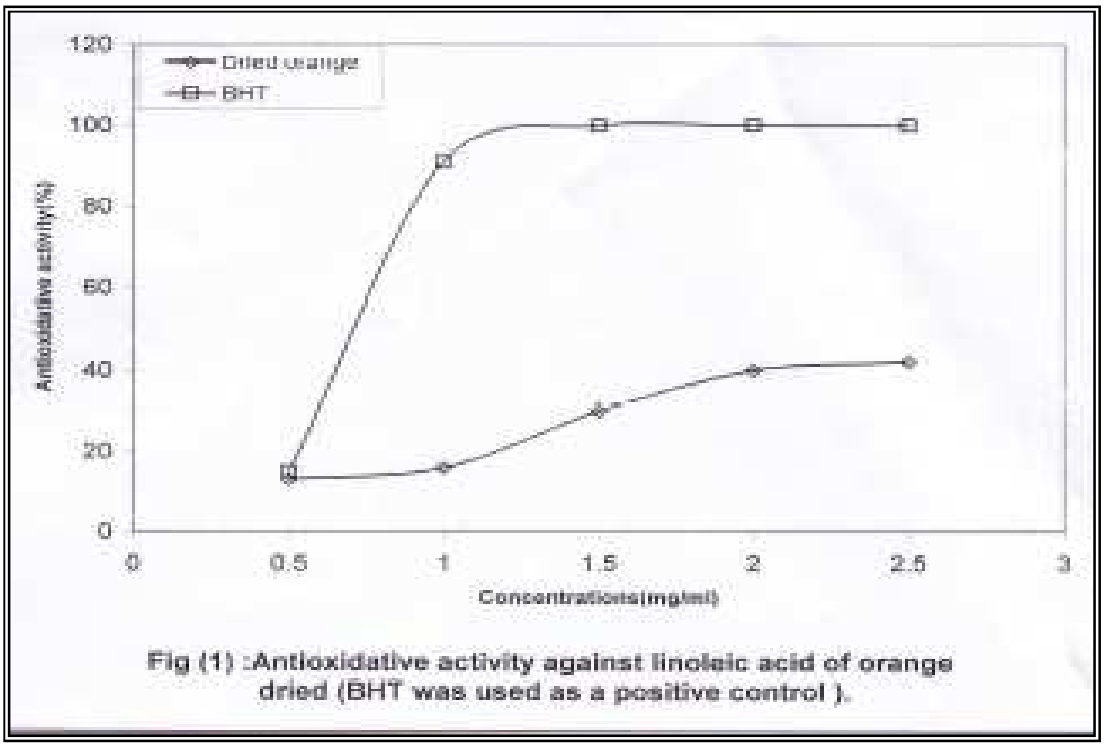


Fig (1) :Antioxidative activity against linolaic acid of orange dried (BHT was used as a positive control).

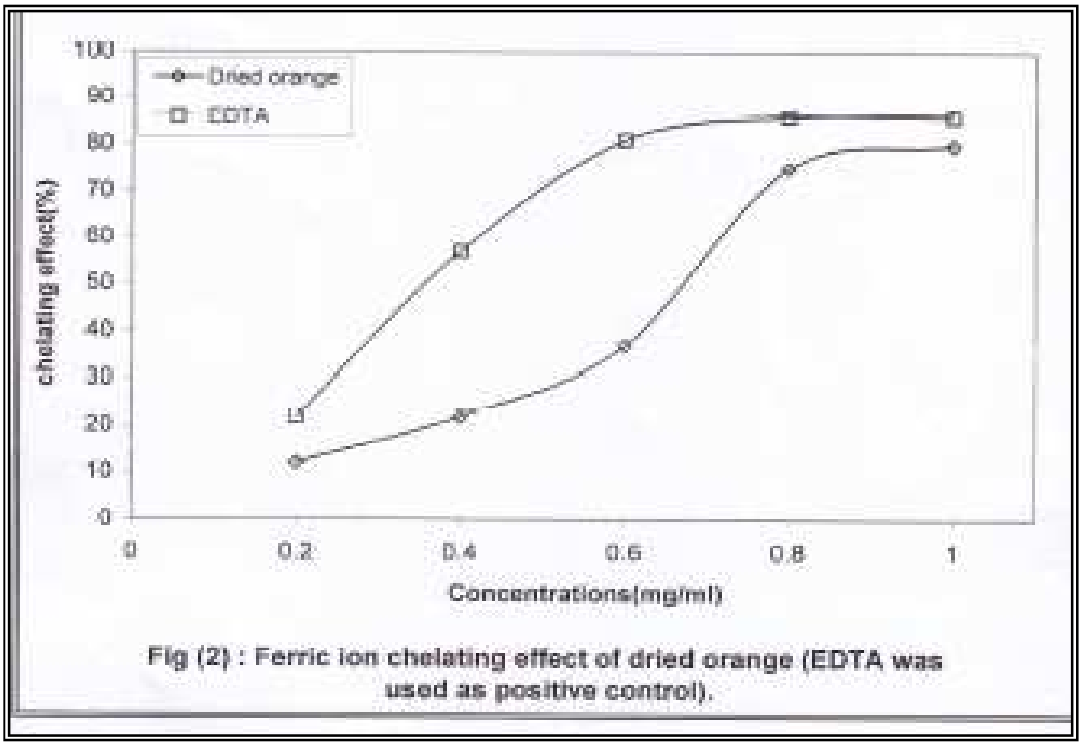
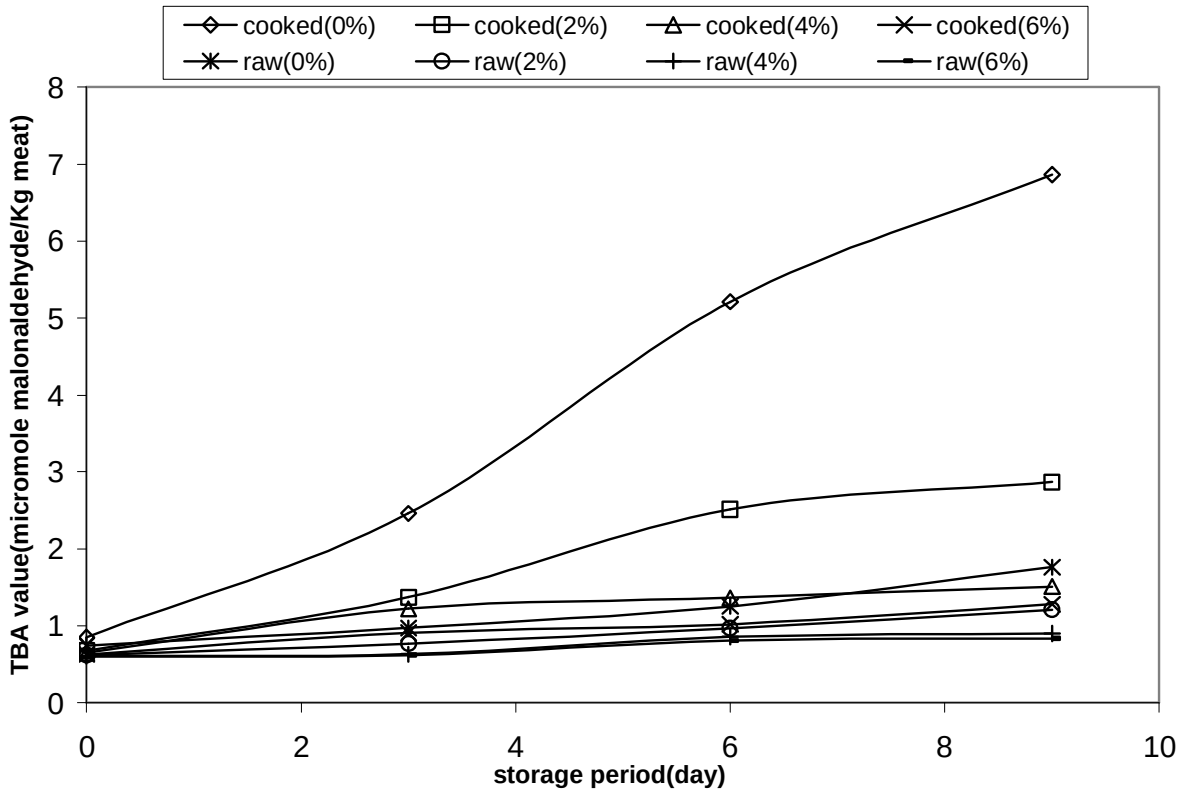
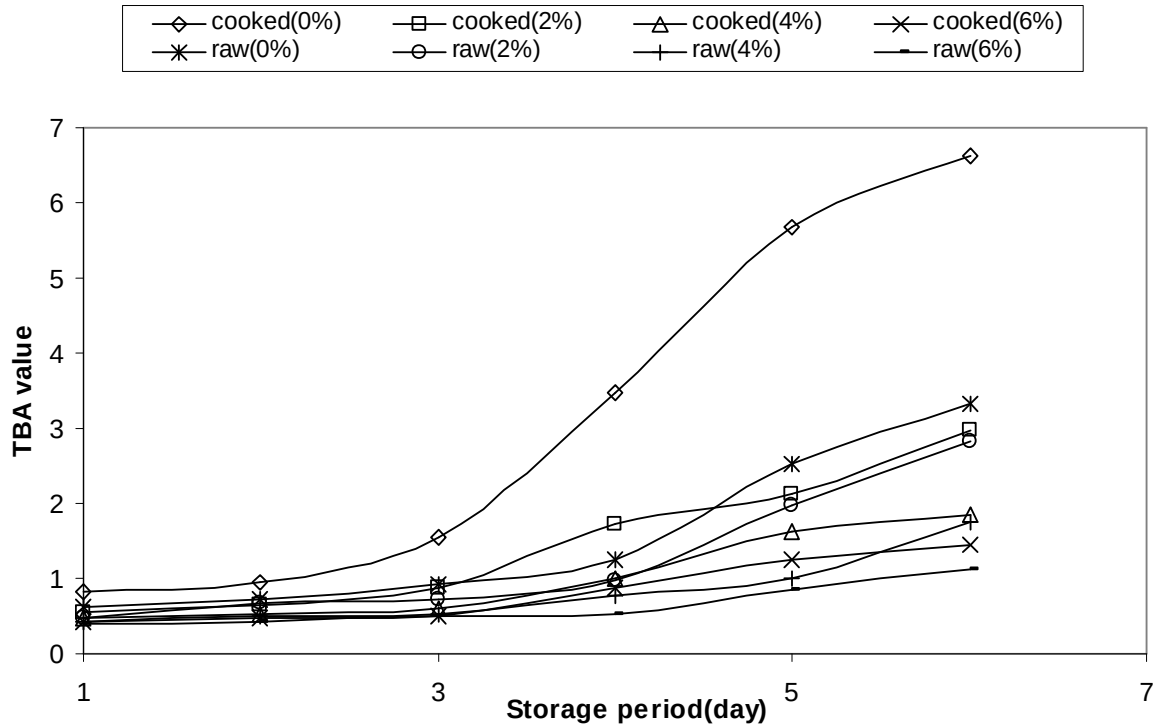


Fig (2) : Ferric ion chelating effect of dried orange (EDTA was used as positive control).

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups (Hatano *et al.*,1989). Dried orange (1mg), 102.5µg gallic acid equivalent of phenols was detected. The phenolic compounds may contribute directly to the antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0gm daily ingested from a diet rich in fruits and vegetables (Tanaka *et al.*,1998). When dried orange incorporated into ground beef and study the antioxidant activity of dried orange by determining the Thiobarbituric acid (TBA), Fig. 3. shows antioxidand activity of dried orange in raw and cooked unfrozen ground beef. The raw samples exhibited non significant differences in TBA number when compaired with the control, however in cooked samples the addition of dried orange at 2%, 4% and 6% significantly reducess the TBA number to 3, 6 and 9 days of storage. There was no significant differences between 2%, 4% and 6% levels. A similar trend was noticed in samples which were frozen, Fig.4. shown that dried orange was uneffective in producing significant differences in TBA numbers in uncooked frozen samples. In the cooked samples dried orang did not produce significantly different TBA values at the first three months of storage (1-3 month) because this periods did not affect the oxidation of the samples and the freezing period was not enough for any changes to occur such as oxidation while the last three months (4-6) produce significantly lower TBA values in the samples containing dried orange at 2% or 4% or 6% levels. The difference between these levels was not significant .According to the results of the above study, it appears that dried orange has antioxidant activity in both meat system and oxidation of linoleic acid system. The present study is show greater oxidation in cooked meat than raw, it is agreement with those found by cross et al.(1979) in which they found cooked beef patties consistently had higher TBA numbers than uncooked. Since there was no significants differences in effectiveness as an antioxidant between levels 2%, 4% and 6% dried orange, it is possible that lower level could be as effective as the ones tested.



Fig(3) : Effect of dried orange on TBA value in unfrozen ground beef .



Fig(4):Effect of dried orange on TBA value in frozen ground beef .

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الفعالية المضادة للأكسدة لمسحوق البرتقال المجفف

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الخلاصة

درست الفعالية المضادة للأكسدة وقابلية ربط ايون الحديديك والمواد الفينولية الكلية في مسحوق البرتقال المجفف، والحالة (BHT)قدرت الفعالية المضادة للأكسدة لحالتين الأولى أكسدة حامض اللينوليك مقارنة بمضاد الأكسدة الصناعي الثاني هي استخدامه في اللحم المفروم، استخدم المسحوق بثلاثة تراكيز 2% ، 4% و 6%. خزنت نماذج اللحم (مطبوخة وغير مطبوخة) في الثلاجة بدرجة حرارة 5م لمدة 9 أيام وخزنت نماذج أخرى في المجمدة بدرجة (−18 ± 2مْ) لمدة 6 ، كما اظهر قابلية ربط (BHT)اشهر. أظهرت النتائج أن لمسحوق البرتقال المجفف فعالية مضادة للأكسدة مقارنة مع جيدة لايون الحديديك وعند متابعة قياس قيمة حامض الثايوباربيتوريك أظهرت النتائج أن استخدام المسحوق بالتراكيز 2% ، 4% و 6% قلل قيمة الحامض بمعنى ذلك أن المسحوق قلل من التزنخ لنماذج اللحم سواء المخزونة بالتبريد أو التجميد كما لوحظ أن استخدام تراكيز مختلفة لم يؤثر معنوياً على قيمة حامض الثايوباربيتوريك.