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INFLUENCE OF DIFFERENT PROCESSING TECHNOLOGIES ON THE ANTIOXIDANT ACTIVITIES OF STRAWBERRIES AND BITTER ORANGE PEELS

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ABSTRACT

The antioxidant activities of both strawberries and bitter orange peels were investigated using DPPH-ESR system and conjugated diene methods. The obtained data showed both excellent antioxidant and antiradical activities of their freeze dried extracts. Bitter orange peels showed also an excellent thermal stability, followed by strawberries. Their methanolic extracts were furtherly fractionated by using GC-MS technique to identify the phenolic compounds responsible for the antioxidative properties of these previously studied materials. It is suggested that bitter orange peels could be applied as a safe natural antioxidant thereby an additional by- product could be achieved. Various methods for pretreatment and drying were tested and the most suitable processing conditions were blanching (10s) and vaccum dehydration at 40^{o}C for 7 hrs, then ground into a fine powder.

Osmotic pretreatment and vaccum dehydration were also proved their applicability to be used for preserving the components responsible for the antioxidant activities in strawberries.

Keywords: antioxidant activity, antiradical activity, bitter orange peels, strawberries, osmotic pretreatment, vaccum dehydration, natural antioxidants.

INTRODUCTION

Epidemiological studies have consistently shown that consumption of a diets high in fruits and vegetables may contribute to maintance of both health and possible risk for chronic diseases, such as coronary heart diseases, cataract, cancer diabetes and alzheumur diseases (Temple 2000) and (Willet 2002).

Strawberries contain high levels of antioxidant compounds which provide protection against harmful free radicals and have been associated with lower incidence and mortality rates in cancer and heart diseases, in addition to number of other health benefits (Gey 1990). It was shown by (Wang and Jias 2000) and (Wang and Lin 2000) that strawberries have high oxygen radical absorbance activity against peroxyl radicals (Roo). Superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2) and their antioxidant activities were different among varieties. However, the differences that are already taken place in the antioxidant activities between cultivars may be related to genotype (Minoggio *et al.*., 2002, Howard *et al.*., 2001), growing season, maturity at harvest, environmental stress and other factors (Kirakosyan *et al.*., 2004).

Citrus processing by – products like peels, represent a rich source of naturally occurring flavonoinds. The peels which represent roughly half of the fruit mass contain the highest concentration of flavonoids. (Manthey and Grohmann 2001). Citrus were found to have a good total radical antioxidative potential compared to α. Tocopherol and BHA (Taiwo *et al.*., 2003). Currently, there is an interest in the recovery of citrus phenols as a functional food ingredients with targeted pharmacological endpoints (Manthey, 2004).

Although, there are several mechanisms of antioxidants due to the reactivity of the phenol moiety (Hydroxyl substitute on the euromatic ring). Antioxidant activity is believed to be radical scavenging via hydrogen atom donation. Other, established antioxidant, radical quenching mechanisms are through electron donation and singlet oxygen quenching (Shahid and Wansundara 1992). Substituents on the aromatic ring affect the stabilization and therefore affect the radical – quenching ability of these phenolic acids. Different acid therefore, have different antioxidant activities. (Chalas *et al.* 2001).

Therefore, a collaborative teamwork has been initiated between Egypt and Turkey to study the differences that might have been found, in these naturally occurring antioxidants derived from both strawberries and bitter orange peels. So, this paper aimed to; fractionate the phenolic compounds in both Turkish and Egyptian samples that are responsible for the antioxidant activities, using G-C-mass spectrum analysis, to identify the main components that are responsible for the antioxidative activities. The osmodehydration process has been carried out to help in maintaining the antioxidant activities of strawberries, and their contents. Finally, bitter orange peels have been studied to be used as a natural antioxidant powder in place of the synthetic ones.

MATERIALS AND METHODS

1.Materials:

Both Egyptian and Turkish strawberries ***fragaria x ananassa*** as well as bitter oranges ***citrus aurantium*** were obtained from the local markets. Turkish strawberry fruits and peels were taken from bitter orange were brought in Egypt, as a freeze dried packed in a vaccum sealed pouches with negligible losses in antioxidant activities (0.5-1%).

2.Methods:

2-1. Sample preparation:

All the previously mentioned samples were extracted using the method described by (Garcia *et al.* 2004). The extraction solution was water: methanol: acetone (8:1:1). These samples were lyophilized and kept at -20^{o}c till used.

2.2. Measurements of the antioxidative properties:

2.2.1. Antioxidant activity:

The antioxidant activity was measured by applying the conjugated diene method using pure sunflower oil (Fu *et al.* 2001). A_{234} was taken as an indication of the course of oxidation using BHT as a control assuming it has

100% activity. The antioxidant activity was evaluated according to the following equation:-

AOA%=(ΔA_{234} control- ΔA_{234} sample)/(ΔA_{234} control)x100…….(1)

2-2-2. Scavenging effect of picryl radical (DPPH) using ESR technique:

Extracts of the studied samples were prepared as described previously. 4ml were mixed with 1ml of methanolic solution containing DPPH 0.2μ as described by (Brunet *et al.* 2005). The resulted spectra were recorded on an ESR, electron spin Bruker-Alex-sup 5000 operated at λ-band frequency. The ESR spectrometer set at the following conditions. 2480 at 4460 G^+ magnetic field, 0.001 field modulation amplitude. The anti-radical activity was defined as

AA% = 100. (Ho-Hs)/ Ho…….. (2)

Where

Ho: Highest of the second peak in the ESR spectrum of DPPH free radical of the blank.

Hs: Highest of the second peak in the ESR spectrum when the extract was added to DPPH free radical.

2-3. Thermal stability of the antioxidants:

Extracts were subjected to heating at 90^{o}c in a water bath at a variable reaction times fixed at 30 and 60 and 90 mins. The residual antioxidant activity of the extracts was measured by the conjugated diene method as described later. The decline in the antioxidant activity against reaction time (min) was studied kinetically by calculating the reaction rate as a slope derived from the linear regression analysis as mentioned by (Indrawati *et al.* 2004).

2-4. Phenolic compounds:

2.4.1. Using Folin-Ciocaltu method:

Total phenolic compounds in the previously prepared extracts were determined using the Folin-Ciocaltu method. Gallic acid was used as a standard (Singleton and Rossi 1965).

2.4.2.GC-MS analysis:

The methanolic extracts of the studied samples were fractionated for their phenolic compounds. The samples were prepared by using small cartridge packed with octadecylsilan (C18) column. The solid phase was firstly conditioned with 5 ml methanol, then 5ml of the extract was passed through the cartidge under vaccum and the methanolic extract of the studied samples were injected into GC model “trace GC 2000” with the following conditions; initial temp 48^{o}c, final temp 250^{o}c with a rate of 5^{o}c.min^{-1} and flow rate 1 ml.min^{-1}. Capillary column DB-5 (phenyl methyl polysiloxane) was used. Electron ionization at 70ev was applied as described by Smolarz (2001). The phenolic compounds in the methanolic extracts were identified using SSQ- 700 finign at mass range 40-400 and scan time 5 mins.

2.5. Applications:

2.5.1. Using bitter orange peels as a natural antioxidant:

To study the suitability of using this material as a natural antioxidant, the following procedures were used as described by (Wolf and Lin 2003). Bitter orange peels, were firstly blanched for 10s to inactivate the potentially active enzyme that may lead to problems with these powders during storage,

then they were dried at 40^{o}c under vaccum for 7hrs before they ground into fine powders. Antioxidant activity, free radical scavenging activity and total phenolic compounds were determined as described, previously.

2.5.2. Osmotic dehydration of strawberries:

To maintain the natural antioxidants, that were found in strawberries, osmotic dehydration pretreatment was suggested and applied as described by (Taiwo *et al.* 2003) using 60% sucrose solution. Strawberries were divided into an equal medium size halves. The ratio 1:10 of the fruit samples to sucrose solution was used. These halves were kept in contact with sucrose solution for 24h period at room temperature, till weight reduction reaches 30-50%. Finally they were vaccum dehydrated at 40^{o}c and 0.3 mbar for 7 hrs.

2.6. Statistical Analysis:

In all the described methods, data were reported as an average of three replications + standard error. T-test method was applied to test the significance of the data.

RESULTS AND DISCUSSION

1. Antioxidative properties of strawberries and bitter orange peels

In this study, two different techniques were applied for measuring the antioxidative activities. The first one is based on the UV technique which measured the hydroperoxides and the conjugated dienes and trienes (Fu *et al.* 2001). The other one is based on examining directly, free radical production and its inhibition by antioxidant using highly sensitive electron spin resonance (ESR) spectroscopy, snice this method is nearly the only technique that directly measure the free radicals; (Brunet *et al.* 2005).

Fig(1), showed the changes, that occurred in the antioxidant activities of the suggested extracts. The data showed non statistical difference between the varieties obtained from both Egypt and Turkey ($p<0.05$). Strawberries extracts showed the highest level of antioxidant activities followed by bitter orange peel extracts. They gave 95.14% and 73.74% when 25 mg. ml^{-1} were used respectively. Most of these antioxidant activities could be related to the presence of phenolic compounds and anthocyanins pigments, which the studies revealed their contribution to the antioxidative properties of these fruits (Eniband *et al.* 2004).

DPPH free radical scavenging activities of the studied extracts using ESR are indicated in (Figs 2 and 3). These figures showed an excellent scavenging activities of strawberries and bitter orange peels, their values were approx. near 96%. Both the obtained two varieties showed the same pattern. These results were confirmed by the study of (Wang and Lin 2000) who noticed that the difference in antioxidant activity and phenolic compounds among the strawberry varieties were minimal.

Concentration up to 25 mg.ml^{-1} reduces all DPPH radical molecules. i.e, the ESR signal vanishes, indicating an excellent antioxidant activity. Obviously, there were more antioxidant components present in these studied extract, which could act rapidly with DPPH and reduce almost all DPPH-radical molecules.

Fig.(1): Changes in the antioxidative activities of both bitter orange peels (A) and strawberries (B) as affected by the varieties and different processing conditions.

Fig.(2): ESR spectra of the bitter orange peels methanolic extracts.

Fig.(3): ESR spectra of the strawberries methanolic extracts

However, it is important to recognize that ESR spectra of particular spin adduct have unique characteristics, they are dependent on the specific spin trap used and the free radical trapped, serving thus as sensitive and specific markers of the presence of a particular free radical species; (Brunet *et al.* 2005). The relative intensity of the free radical formation can be determined, because ESR spectroscopy signal is directly related to the concentration of the spin adduct. The highest of the peak in the spectrum in directly related to the number of radical molecules in the accumulating system.

Natural phytochemicals such as phenolic compounds found in the studied extracts commonly involve an aromatic ring as part of the molecular structure with one or more hydroxyl groups. They can acts as antioxidants as their extensive conjugated II electron system allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals (Evans *et al.* 1996). The antiradical efficiencies based on this mechanisms are the typical for different phenolic acids and flavoniods, whose presence has been existed in these two extracts.

3.2. Influence of blanching and vaccum dehydration on the antioxidant activity of bitter orange peels:

Fig(1.A), indicated the changes, that are taken place in the antioxidant activities of bitter orange peels after blanching for (10s) and vaccum dehydration (0.3 mbar 40^{o}c). The antioxidant activities of these products were lowered from 74 to 62% when 25 mg.ml^{-1} extracts were used.

The free radical scavenging activity marked as an AA% was also affected; (fig. 2). The antiradical activity was lowered to be 59.1%, when the same concentration was used. From the previously, mentioned results it could be concluded that, bitter orange peel powder could retain 82% of their phenolic compounds, but their antioxidative properties are affected; i.e. that is mean that, there is other components might be responsible for the free radical scavenging activity. Table (1) summaries these changes, since their EC_{50} values are becoming greater. (from 12.7 to 17.17mg). This product is considered as a waste product, so it could be added in gm levels to attain the desired antioxidative properties.

3. Osmotic dehydration of strawberries

Osmotic dehydration before vaccum air drying has been suggested to yield an intermediate moisture products of improved stability requiring less drying time (Alwarez *et al.* 1995). Also this technique has proved to be useful to improve the quality of a delicate tissue such as that of strawberry (Torreggiani and Bertalo 2001). The Osmo-dehydrated strawberry halves showed also pigment retention significantly higher than that observed in the fruits treated by conventional methods (Eric and Schubert, 2001).

As indicated in Figs (1b and 3) the osmotic pretreatment method lowered the strawberry antioxidant activity from 90.90% to 72% and the antiradical activity from 97.08 to 72.8%. This observation could be related to the changes that occurred in the colour of the osmotic solution, during osmo dehydration, due to the leaching of pigments (anthocyanogenic compounds). These anthocyanogenic compounds are known of their contribution to the antioxidant properties of the most of colorful foods such as strawberries.

The leaching of the pigments in sugar osmotic solution, creat an aqueous extracts contain the potent polyphenolic antioxidants such as anthocyanin and tannis (Wang *et al.* 1996), thereby additional losses in antioxidant activities were observed, consequently their Ec_{50} values are arised to attain the desired antioxidative properties.

Table (1): Changes in the total phenolic compounds as a function of antioxidant activities measured as AOA%* and AA%**

Raw materials	****EC_{50}	***Total phenolic compounds mg gallic acid eq	AOA%	AA%
Strawberries				
Turkish freeze dried	12.7	10.50+2.4	95.14+3.6	98.12+2.7
Egyptian freeze dried	12.9	9.66+0.5	90.90+5.2	97.08+3.9
Osmotic dehyderation	17.17	7.63+1.3	71.60+2.9	72.80+5.2
Bitter orange peels				
Turkish freeze dried	13.04	9.99+3.1	73.74+4.1	95.83+1.7
Egyptian freeze dried	13.03	9.97+2.9	72.29+5.2	95.80+2.9
Blanching+vaccum dehyderation 40^{o}C 7 hrs	21.15	8.23+1.3	60.96+8.1	59.10+3.1

*** AOA% antioxidant activity using conjugated diene method.**
**** AA% anti-radical activity using DPPH-ESR method.**
***** Total phenolic compound were estimated in 25 mg.ml^{-1}**
******EC_{50}: is the effective concenturation that required to attain 50% AA%**
Data are an average of three replicates+ SE.

4. Thermal stability

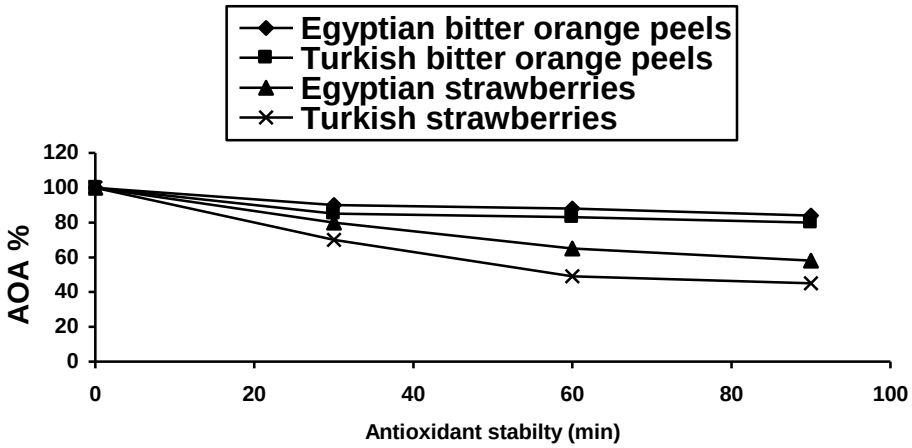
Fig.(4) showed the thermal stability of these studied extracts. The data were expressed in terms of first order reaction rate constant. The K values (min^{-1}) were tabulated in the same figure. Both the Egyptian and Turkish orange peels extracts showed the same lowest k values (0.0022 min)$^{-1}$ indicating that they have the most stable thermal stability followed by strawberries extracts, they were (0.0068 and 0.0095 min^{-1}) in both the Egyptian and Turkish varieties.

Such these remarkable stabilities concerning bitter orange peels; suggested their potential application as an antioxidant powder to be used efficiently to retard oxidative rancidity, as suggested by (Wolf and Lin 2003). They indicated that peels from fruits could be used as a natural antioxidants in place of the synthetic one. The information shows that their extracts can be used as an antioxidant in food and medicinal preparation (Angnostopoulou *et al.* 2006).

5. Total phenolic and GC-mass:

Table (1) illustrated the total phenolic compounds of the studied methanolic extracts. The obtained data showed a strong correlation coefficients (R^2) between the total phenolic compounds and antioxidant activities measured as AOA% and AA%. They were 0.9153 and 0.8271 in case of strawberries and 0.998 and 0.999 in case of bitter orange peels, respectively. Other, studies have indicated that phenolics are responsible for

the major portion of total antioxidants in strawberries. Strawberries contain numerous phenolic compounds, and not all the cultivar may contain the same phenolic profile, differences in these profiles may subsequently result in complex changes in antioxidant activity of these bioactives (Macheix *et al.*., 1990). Regarding to orange peels; (Manthey 2004) have concluded that there is a lot of structurally diverse phenols in orange peels that could be acted as an important radical scavenger.



Variety	K(min^{-1}) ± SE
Egyptian bitter orange peels	0.0022 ± 0.02
Turkish bitter orange peels	0.0022 ± 0.04
Egyptian strawberries	0.0068 ± 0.06
Turkish strawberries	0.0095 ± 0.016

Fig.(4): Antioxidant stability of the methanolic extracts recovered from strawberries and bitter orange peels .

Figs (5 and 6) showed the GC- mass chromatogram of the studied methanolic extracts. Results showed that most of these compounds have antioxidative properties since they are terpens, alcohol hydrocarbons and ester with double bonds. All excert this property as a reducing agents, oxygen quencher and metal chelating agents (Robbins 2003)and (Emara and Abdel-Kader 2004).

One of the most dominant compounds that are appeared in the chromatograms of bitter orange peels are the presence of desulphosinarigin which is appeared to be one of the narigin derivatives (Belajova and Suhaj 2004). It is appeared in the Turkish varieties, whereas in the Egyptian varieties limonene was found. There is no difference in both the Turkish and Egyptian strawberries varieties since the most dominant compounds found were DL limonene and furancarboxyaldehyde. Other studies by (Carmona *et al.*., 2005) suggested that phenolic compounds and antioxidant activities are strongly dependent on genotype, rather than climate or season.

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Conclusion:

This study has indicated the suitability of applying bitter orange peels as a source of natural antioxidants thereby an additional by- product is achieved. Osmotic pretreatment was also pointed out as an excellent method for maintaining the antioxidant activities in a sensitive fruits like strawberries. Strawberries preserved by this technique contained a substantial amounts of anthocyanins and phenolic compounds that can be acted as a strong antioxidants. GC-mass analysis have proved that most of the phenolic compounds detected exerted antioxidant activity.

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تأثير بعض المعاملات التكنولوجية على درجة النشاط المضاد للأكسدة فى كل من الفراولة وقشور الجريب فروت

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أجريت هذه الدراسة بالتعاون مع الجانب التركي لمعرفة تأثير الاختلافات فى الأصناف المختلفة المنزرعة من الفراولة والجريب فروت فى كل من مصر وتركيا على درجة النشاط المضاد للأكسدة والمواد الفينولية المسئولة عن ذلك. ولتحقيق هذا الغرض تم فصل هذه المكونات باستخدام جهاز كروماتوجرافيا الغاز بغرض تحديد المكونات المسئولة عن النشاط المضاد للأكسدة وفى نفس الوقت استخدمت طريقة التجفيف الأسموزى والتجفيف تحت تفريغ بغرض الحصول على مسحوق من الجريب فروت ودراسة مدى إمكانية استخدامه كمادة مضادة للأكسدة بديلاً عن تلك المواد الصناعية المستخدمة، هذا وقت أثبتت الدراسة أيضاً القدرة العالية للمستخلص الكحولي من الفراولة والجريب فروت أن له قدرة عالية كمادة مضادة للأكسدة بنسبة تقترب من ٩٦% وذلك عند قياس النشاط المضاد للأكسدة وذلك باستخدام جهاز "ESR" واستخدام مادة "DPPH" كشق حر. وقد أثبتت الدراسة أيضاً أن أفضل الطرق المستخدمة لحفظ الفراولة بغرض المحافظة على النشاط المضاد للأكسدة هي قطعها نصفين وتعريضها لكل من التجفيف الأسموزى كمعاملة مبدئية ثم بعد ذلك التجفيف تحت تفريغ حيث كان لها أكبر الأثر فى الحصول على منتج ذو درجة رطوبة متوسطة ذات درجة ثبات عالية كذلك كانت لهذه الطريقة أكبر الأثر فى الحفاظ على صبغة الأنثوسيانين الموجودة فى الفراولة والتي لها بالطبع دور أساسى فى النشاط المضاد للأكسدة.

أما بالنسبة لقشور الجريب فروت فقد أثبتت التجارب التى أجريت أن أفضل طريقة لمعاملتها هى عملية السلق لمدة (١٠ث) ثم بعد ذلك التجفيف تحت تفريغ (٠,٣ ميجا بار على ٤٠°م للحصول على مادة مضادة للأكسدة طبيعية يمكن استخدامها بعد طحنها حيث أثبتت التجارب التى أجريت أن لها درجة ثبات عالية.

وباستخدام كروماتوجرافيا الغاز فقد أثبتت التجارب وجود مادة desulphonarigin والذى يوجد فى الأصناف التركية مقارنة بوجود مادة الليمونين limonene فى الأصناف المصرية فى حين أنه لم يكن هناك أى اختلافات ظاهرة فى كل من أصناف الفراولة المنتجة من مصر أو تركيا حيث كانت أهم المواد الموجودة هى limonene, furancarboxyaldehyde وقد أثبتت أن الاختلافات فى درجة النشاط المضاد للأكسدة يكون لنوع الصنف تأثيراً عليها أكثر من ظروف المناخ أو الموسم الذى تم أخذ العينات منه.