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Antioxidant effects of strawberry fruits at two phenological stages

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ABSTRACT

Background & Aim: Strawberry (*Fragaria vesca* L.) is a plant belonging to the Rosaceae family with medicinal properties, including antioxidant activity. The present study aimed at investigating the antioxidant properties of strawberry.
Experimental method: To examine the antioxidant effect, fruit extracts at two phonological stages of growth (ripe and unripe strawberry) were prepared. In addition, four different solvents, including 80% ethanol, 80% methanol, acetone and distilled water were used for the preparation of plant extracts. In total, eight different plant extracts were prepared and their properties comparatively studied. To study the antioxidant effect, potassium ferricyanide and reducing power determination method were used.
Results & Discussion: Results indicated that strawberry fruit had antioxidant effects in both of two stages. That is due to the presence of the higher pigments and phenolic compounds in ripe fruit than unripe fruit.
Recommended applications/industries: The results of present indicated the red fruit had the highest antioxidant activity.

1. Introduction

Nowadays, the advantage of using medicinal drugs is obvious for everyone, while chemical drugs have many side effects. In current world, the human environment is full of mutative materials that are the agent of many diseases such as cancer. Plants are very effective in preventing such diseases with trapping free radicals and having antioxidant effects (Zeng *et al*., 2010).

Strawberry (*Fragaria vesca* L.), belongs to the family Rosaceae, is widely consumed, both as fresh fruit and as an ingredient in processed products. It is a very rich source of bioactive compounds including vitamin C, E, β-carotene, and phenolic compounds (phenol acids, flavan-3-ols, flavonols, and anthocyanins) (Oszmianski & Wojdylo, 2009). The major phenol compounds are procyanidins, ellagitanins, catechin, and *p*-coumaroyl esters (Aaby *et al.*, 2005).

Strawberries are unique with highly desirable taste, flavour, excellent dietary sources of ascorbic acid, potassium, fiber and other secondary metabolites and also simple sugar sources of energy (Wang & Galletta, 2002). Various beneficial biological effects of strawberry fruits consumption have been documented, such as an increase of the serum antioxidant capacity in humans (Cao *et al.*, 1998), anti-carcinogenic activity (Carlton *et al.*, 2001; Wedge *et al.*, 2001), anti-thrombotic effects (Naemura *et al.*, 2005), etc.

The strawberry is one of the most commonly consumed berries. Its high nutritional value has long been correlated to the relevant content of antioxidant micronutrients, such as vitamin C and folate, and more recently to the high variety and content of antioxidant polyphenolic constituents, such as flavonoids, hydrolysable and condensed tannins, and phenolic acids (da Silva Pinto *et al.*, 2010; Seeram *et al.*, 2006). Glucose is the most common sugar substituent in

strawberry, but rutinose, arabinose and rhamnose have also been tentatively identified. Other minor anthocyanins, include acylated derivatives with the following organic acids: malic, malonic, succinic, or acetic acids (Lopes-da-Silva *et al.*, 2007).

Significant increases in the plasma total antioxidant capacity (TAC) have already been reported after acute intake of strawberries. In addition, anti-haemolitic effects of strawberry extracts have been recently demonstrated *in vitro*, revealing that part of the antioxidant properties of strawberry bioactive compounds could lie in their localisation within cell membranes. A significant increase in fasting plasma TAC and in serum vitamin C concentrations were progressively observed during the period of strawberry supplementation. An enhanced resistance to haemolysis was also observed in both AAPH-treated and untreated erythrocytes, collected during and after the period of strawberry consumption. The results obtained in this work suggest that regular consumption of antioxidant-rich strawberries may exert an improvement on the plasma antioxidant status (Tulipani *et al*., 2011). In addition, epidemiological studies have shown that high fruit and vegetable consumption has health benefits in the prevention of chronic diseases (Ness & Powles, 1997; Steinmetz & Potter, 1991). Several studies have suggested that the phytochemical content and antioxidant / free radical scavenging effect of fruits and vegetables contribute to their protective effect against chronic and degenerative diseases (Heinonen *et al.*, 1998; Record *et al.*, 2001). Strawberry extracts were found to have higher antioxidant activity, as indicated by the oxygen radical absorbance capacity assay, than extracts from plum, orange, red grape, kiwifruit, pink grapefruit, white grape, banana, apple, tomato, pear, and honeydew melon (Meyers *et al*., 2003).

Increase in the consumption of foods rich in antioxidants, micronutrients and phytochemical has long been proposed as a preventive measure to decrease the risk of several chronic diseases caused by oxidative stress. In particular, the improvement in plasma antioxidant status (and the increase in serum antioxidants) has been recognized as a potential tool to prevent the development of cardiovascular, proliferative, degenerative and other diseases (Kay & Holub, 2002).

Anthocyanin increasing concentrations in ripe strawberries is a well-known phenomenon (Ferreyra *et al*., 2007; Given *et al*., 1988; Wang and Lin, 2000). The main anthocyanin found in strawberries is pelargonidin 3-glucoside, with cyanidin 3-glucoside and pelargonidin 3-rutinoside present as minor components (Gil *et al.*, 1997). Therefore, there is a lack of research evidence from *in vivo* protracted strawberry consumption studies. We carried out a 16-day pilot study where 12 healthy subjects ingested 500 g of antioxidants-rich strawberries daily, and we evaluated the potential effects of fruit consumption on biomarkers of plasma and cellular antioxidant status.

2. Material and Methods

2.1. Plant Samples

Unripe and ripe fruits of strawberry were collected from the strawberry farm in Rasht, northern Iran on April 2012. The fruits were dried in shadow and then were powdered and stored at 25 °C until use.

2.2. Ethanol and methanol extracts of strawberry

For preparation of ethanol and methanol extraction, 1 g autoclaved power of strawberry was added to 10 ml of 80% ethanol and methanol, separately. Then, the extracts were filtered from filter paper. By this way ethanol and methanol extracts of strawberry was produced. The extracts were stored at fridge for 48 h until use.

2.3. Acetone and distilled water extracts of strawberry

For preparation of ethanol extraction, 1 g autoclaved power of strawberry was added to 10 ml of acetone and distilled water, separately. Then, the extracts were filtered from filter paper. By this way acetone and distilled water extracts of strawberry was produced. The extracts were stored at fridge for 48 h until use.

2.4. Control samples

Control samples used in this assay were acetone, distilled water, methanol and ethanol solvents.

2.5. Measurement of fruit antioxidant activity

Reducing power of both extracts of strawberry were measured by method of ferric reducing power. According to this method, the reduction of Fe^{3+} to Fe^{2+} was determined by measuring absorbance of the Prussian blue complex. This method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants. For this purpose,

10% concentrations of distilled water extract, ethanol, methanol, and acetone extracts of strawberry were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml (1%) of potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 min. After 20 min of incubation, the reaction mixture was acidified with 1 ml of trichloroacetic acid (10%). Finally, 2.5 ml of $FeCl_3$ (0.1%) was added to this solution. Distilled water was used as blank and for control. Absorbance of this mixture was measured at 700 nm using a spectrophotometer. Increased absorbance indicates ferric reducing power capability of sample.

2.6. Statistical analysis

Antioxidant assays were repeated three times, and the data were analyzed by statistical software, SPSS (ver. 17) and Excel software. And related graphs were drawn.

3. Results & Discussion

In figures 2a, 2b, and 2c, control, unripe and ripe solvents of strawberry have been shown, respectively. Results indicate that control sample had not antioxidant effect, whereas the fruit had antioxidant activity at both of phenological stages. But in ripe fruit was higher than unripe. Because phenol compounds such as anthocyanins are the higher in ripe fruit. After adding $Fecl_3$, the color of Prussian blue was observed in ripe and unripe extracts (Fig 3b and Fig 3c). And Prussian blue did not observe in control sample (Fig 3c).

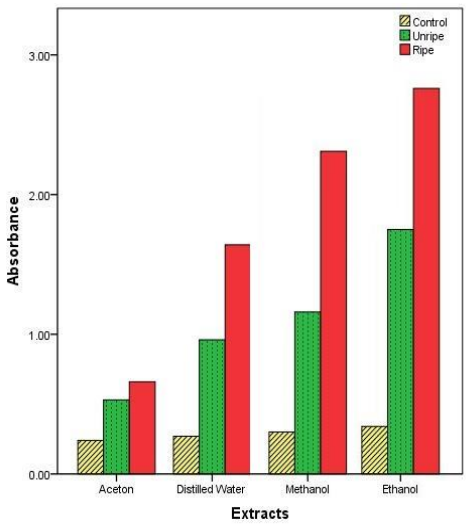
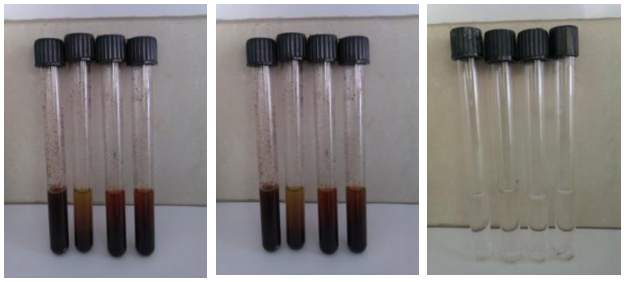


Fig 1. Comparison of the rate of absorbance within four different solvents at 700 nm



c **b** **a**

Fig 2a: From right to left respectively ethanol, methanol, acetone and distilled water of control solvent; **2b:** From right to left respectively ethanol, methanol, acetone and distilled water of unripe fruit; **2c:** From right to left respectively ethanol, methanol, acetone and distilled water of ripe fruit

c **b** **a**

Fig 3a: From right to left respectively ethanol, methanol, acetone and distilled water of control solvent after adding $Fecl_3$; **3b:** From right to left respectively ethanol, methanol, acetone and distilled water of unripe after adding $Fecl_3$; **3c:** From right to left respectively ethanol, methanol, acetone and distilled water of ripe fruit after adding $Fecl_3$

Table 1. Comparison evaluation of antioxidant effects between different extracts at 700 nm

Samples / Extracts	Control	Unripe extract	Ripe extract
Ethanol	0.34	1.75	2.76
Methanol	0.30	1.16	2.31
Distilled water	0.27	0.96	1.63
Acetone	0.24	0.53	0.66

The rate of absorbance was measured at 700nm (Table 1) and comparison graph of control and ripe and unripe extracts was drawn (Fig 1). Among extracts, respectively ethanol, methanol, distilled water and acetone extracts of ripe and unripe fruit had the most effect. The rate of absorbance also was in low level in ripe acetone extracts because effective materials of fruit dissolve very little in acetone solvent.

In aerobic cells, ROS produces during the normal aerobic metabolic process (Chance *et al*., 1979; Halliwell *et al*., 1989). It has estimated that 1-5% of consumed oxygen by cells can produce ROS (Chance *et al*., 1979). In addition, to endogenic production ROS

could be produced by exogenous factors such as ionizing radiation, diet and foreign materials. Oxidative stress could be arise by extra production of ROS or deficiency in antioxidant action or repairing mechanism which causes reversible or irreversible damage to the vital micro molecules of cells such as lipids, proteins and DNA (Davies *et al*., 1987). It has shown that unrepaireble oxidative stress plays the role in several chronic diseases like cancer (Ames *et al*., 1993). Cells have several mechanisms for inactivation of ROS and repair and replacing the damaged molecules to maintain the cell homeostasis (Yu, 1994).

Some studies (Heinonen *et al*., 1998; Vinson *et al*., 2001) have pointed out that strawberry generally possesses a high level of antioxidant activity, which could be linked to the levels of phenolic compounds in the fruit. Wang and Jiao (2000) showed that strawberry juice exhibited a high level of antioxidant capacity against free radical species including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen.

Various beneficial biological effects of strawberry fruits consumption have been documented, such as an increase of the serum antioxidant capacity in humans (Cao *et al*., 1998), anti-carcinogenic activity (Carlton *et al*., 2001; Wedge *et al*., 2001), anti-thrombotic effects (Naemura *et al*., 2005), etc. These beneficial effects have been mostly attributed to the phenolic compounds that are found in large quantities in strawberry fruits (Hannum, 2004). This is especially true for the wild strawberry (*Fragaria vesca* L.) which has a several times higher antioxidant capacity than the average of the most common, cultivated strawberry varieties (Scalzo *et al*., 2005).

Reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species (Gülçin *et al*., 2003). Reducing power of methanol, ethanol, distilled water and acetone extracts of strawberry was investigated by ferric reducing power assay. Antioxidant compounds cause the reduction of ferric (Fe^{3+}) form to the ferrous (Fe^{2+}) form because of their reductive capabilities. Prussian blue-colored complex is formed by adding $FeCl_3$ to the ferrous (Fe^{2+}) form. Therefore, reduction can be determined by measuring the formation of Prussian blue at 700 nm (Chung *et al*., 2002). In this assay, yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power.

As shown in Fig 1, the ethanol and methanol extracts of strawberry showed increased ferric reducing power and following this, distilled water and acetone showed little antioxidant activity. According to results of the present study, ferric reducing power of ripe fruit extract was higher than unripe. In addition, among the extracts, the rate of absorbance in ethanol extract was higher than others. Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Dorman *et al*., 2003).

(Yellow complex) Fe_4 [Fe $(CN_6)]_3$ ⟶ $K_3[Fe(CN_6)]$
(Prussian blue)

In the most of plants, antioxidant activity and trapping the free radicals is due to the phenolic compounds which cause the reduction of Fe^{3+} in potassium ferricyanide. Because there are a lot of hydroxyl group in the structure of phenolic compounds.

4. Conclusion

Our results indicate the extracts from red fruit of strawberry have the highest antioxidant activity that is due to the presence of the higher pigments and phenolic compounds in ripe fruit than unripe fruit.

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