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Free Radical Scavenging Enzymes of Fruit Plant Species Cited in the Holly Quran

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Abstract: The antioxidant properties of extracts of the (16) food items selected from those cited in the Holly Qur'an were evaluated. The analysis include superoxide radical scavenging activity superoxide dismutase, catalase and glutathione peroxidase. All plant samples tested in this study, showed the presence of antioxidant enzymic activity with varying degrees. Superoxide dismutase (SOD), GSH-PX and CAT activities were significantly high in bananas, figs, garlic and ginger, based on the fresh and dry weight. Grapes also showed high activities of GSH-PX and CAT but were low in SOD activity. On the other hand, wheat, gourd and lentils were moderate in their content of GSH-PX and CAT but were high in SOD activity. In general, this study suggest that catalase is a key enzyme in active oxygen detoxification during fruit ripening.

Key words: Holly Qur'an · Radical scavenging enzymes · Fruits

INTRODUCTION

While oxygen is fundamental for the survival of all aerobic organisms, it is subjected to in vivo actvation into toxic forms. The most damaging forms of active oxygen are free-radicals such as superoxide ($O_2^{\cdot-}$) and hydroxyl ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). Active oxygen forms were reported to cause membrane rigidification, peroxidation of membrane lipids, protein denaturation and DNA mutation [1], leading to metabolic and structural dysfunctions and cell death. In order to decrease these biological damage for survival, all organisms have evolved a well integrated antioxidant system. Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically and in combination with each other to protect the cells and organ systems of the body against free radical damage [2]. The antioxidants can be endogenous or obtained exogenously ,as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (G-POD) and the ascobate-glutathione cycle enzymes: ascorbate-peroxidase (AsA-POD), mono and dehydroascorbate reductase (MDHAR, DHAR) and glutathione reductase (GR). Nonenzymatic antioxidants include Vitamin E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), melatonin, carotenoids, natural flavonoids and other compounds. Some antioxidants can interact with other antioxidants regenerating their original properties; this mechanism is often referred to as the "antioxidant network" [3].

Fruits are one of the oldest forms of food known to man. There are many references to fruits in ancient literature. Vedas state that the fruits form the base of the Food of Gods. Fresh and dry fruits are natural staple food of man . They contain substantial quantities of essential nutrients in a rational proportion. They are excellent sources of minerals, vitamins and enzymes. They are easily digested and excise a cleansing effect on the blood and the digestive tract. Human beings have used plants as medicine from the very beginning of time. After various observations and experimentations medicinal plants were identified as a source of important medicine, therefore, treatment through these medicinal plants, began in the early stages of human civilization [4].

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The Holy Quran is one of the reference books describing the importance of plants used for different aliments in various Surahs, (He it is Who sendeth down water from the sky and therewith We bring forth buds of every kind; We bring forth the green blade from which We bring forth the thick-clustered grain; and from the date-palm, from the pollen thereof, spring pendant bunches; and (We bring forth) gardens of grapes and the olive and the pomegranate, alike and unlike. Look upon the fruit thereof, when they bear fruit and upon its ripening. Lo! herein verily are portents for a people who believe). Al-Anaam – Verse (99). The prophet (Sallallaho Alayhi Wasalam) frequently commented upon the nature and value of various food and spices. The purpose of this study is to evaluate the antioxidant enzymes glutathione peroxidase, catalase (CAT) and superoxide dismutase (SOD) of (16) food items selected from those cited in the Holly Qur'an.

MATERIALS AND METHODS

Plant Materials: A total of 16 plant materials (8 fruits, 6 vegetables and 2 types of grains) were chosen from those cited in the holy Qur'an, to conduct this study on antioxidants from natural sources. All raw materials are described in Table 1 with more details below. These food items are commonly consumed in Saudi Arabia and were purchased from local markets in Makkah during 2008 harvesting seasons (because the laboratory studies conducted over two years during the summer only). They were stored in the refrigerator (at 5°C) except for onion, garlic, lentils and wheat, which were kept at room temperature, until processed, within two days.

Sample Extraction: The extraction procedure was according to Huang *et al.* [5] with a slight modifications, samples were extracted with 0.1 M cold potassium phosphate buffer for 6 hr at 4°C. Cold centrifugation was used at 4°C to yield clear solution without affecting enzymes.

Preparation of Freeze-Dried Extracts: Fifty grams of ground plant materials were lyophilized in vacuum flask for 48 hr at -62°C in a freeze-dryer. The resultant dried samples were weighed, then powdered in food blender and stored in plastic screw-cap bottles at -20°C until analyzed.

Catalase Activity: Catalase assay kit supplied by Sigma-Aldrich Chem (St. louis, U.S.A) according to to the method of Kar and Mishra [6] provides a direct UV assay following the decrease in absorbance of hydrogen peroxide at 240 nm for catalase samples that do not contain UV interfering substances. one unit of CAT will decompose 1imol of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 25°C at a substrate concentration of (10 Mm) hydrogen peroxide.

Glutathione Peroxidase Activity: Glutathione peroxidase assay kit supplied by Sigma-Aldrich Chem (St. louis, U.S.A) using the method of Tappel [7]. It is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GSH-PX, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase and NADPH. The decrease in NADPH absorbance measured at 340 nm during the oxidation of

Table 1: Description of the 16 studied plant material samples

	Scientific name	Common name	Source	Character	Part used
	Fruits				
1	*Musa cavendish*	Banana	Jezan	seasonal	Pulp
2	*Phoenix dactylifera*	Dates	Madina	seasonal	All
3	*Ficus* sycomorus	Figs	Tabuk	seasonal	All
4	*Vitis vinifera*	Red Grapes	Tabuk	seasonal	Pulp+ Peel
5		White Grapes	Taif	seasonal	
6	*Olea europaea*	Black Olives	Egypt	Year round	Pulp+ Peel
7		Green Olives	Egypt	Year round	
8	*Punica granatum*	Pomegranate	Taif	seasonal	Pulp
	Vegetables				
9	*Cucumis sativus*	Cucumber	Tabuk	Year round	All
10		Snake Cucumber	Taif	seasonal	All
11	*Cucurbita pepo*	Gourd	America	Year round	Pulp
12	*Allium sativum*	Garlic	Baladi	seasonal	Pulp
13	*Zingiber officinale*	Ginger	China	Year round	Pulp
14	*Allium cepa*	Red Onions	Tabuk	Year round	Pulp
	Grains				
15	*Lens culinaris*	Orange Lintel	Turkey	Year round	All
16	*Triticum durum*	Wheat	Qassim	Year round	All

NADPH to $NADP^+$ is indicative of GSH-PX activity. Enzyme activity was expressed as the formation of 1µmol of $NADP^+$ per minute at pH 8.0 at 25°C.

Superoxide Dismutase Activity: Superoxide dismutase assay kit supplied by Cyman Chemicals U.S.A., utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine using the method of Monk *et al.* [8]. SOD Activity expressed as Unit ml $extract^-$ (U/ml). One SDO unit was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Measurements of Protein Concentration: Protein concentration was determined spectrophotometrically at 280 nm using UV-visible spectrophotometer by the method of Plummer [9].

Statistical Analysis: All data were expressed as means ± standard deviation of (6n) measurements. Correlation between the antioxidant activity and total phenolic contents was carried out using the correlation and regression in the Excel program (Microsoft Excel v. 2007). One-way analysis of variance (ANOVA) was applied using (SPSS v.15).

RESULTS

The antioxidant enzymes of fresh and dry plant samples are shown in Figure 1. Dry samples of bananas and figs showed the highest values of all the three antioxidant enzymes. Banana fresh sample (wet weight) showed only a high SOD activity, while fresh figs possessed moderate activities of all three enzymes. Fresh garlic showed higher CAT and SOD activities and

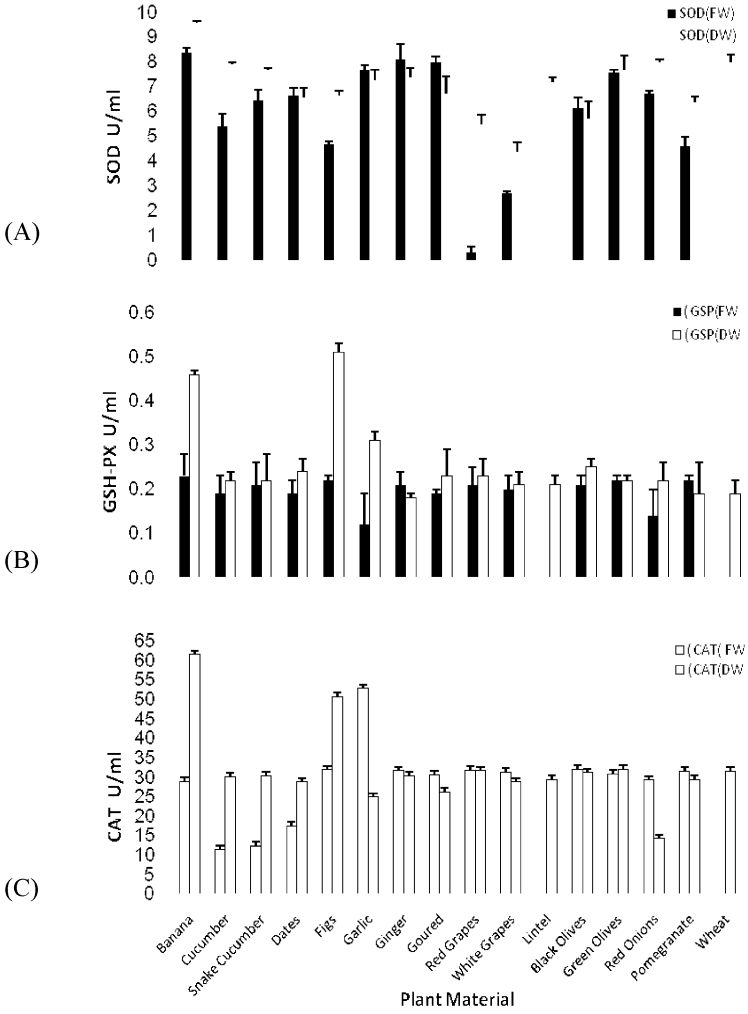

Fig. 1: Activities of superoxide dismutase (A), glutathione peroxidase (B) and catalase (C) enzymes in wet and dried extracts. Data are displayed with mean ± SD (bars) (6n)

low GSH-PX activity, while the dried samples had moderate values of all enzymes. Superoxide dismutase was detected to be very low in fresh sample of red grapes fruit in comparison to the other two antioxidant enzymes.

DISCUSSION

It is well established that free radicals are generated as byproducts of normal metabolism, especially under pathological conditions and a decrease in the efficiency of their removal results in increasing oxidative stress [10]. In order to decrease these biological damages for survival, all organisms have evolved a well integrated antioxidant system, including enzymatic and non enzymatic components [2]. Moreover, apart from being a defensive system against the formation of free radicals in the cell, the SOD, CAT and GSH-PX enzymes are part of a well defined enzymic system, which is involved in the ripening process of fruits as well [11]. Therefore, we determined the activities of SOD, CAT and GSH-PX enzymes in full mature fruits to determine whether or not these enzymes play a role in the radical scavenging activity of each of the plant sample tested.

GSH-PX destroys H_2O_2 by an NADPH-dependent reduction of GSSG to GSH, which plays an important role in regulating fruit ripening. It has also been suggested to be a rate limiting enzyme for defense against ROS toxicity in higher plants. Reduced and oxidized glutathione (GSH and GSSG) content increased from young fruit stage to early stage of fruit color turning [10].

All plant samples tested in this study, showed the presence of antioxidant enzymic activity with varying degrees. Superoxide dismutase, GSH-PX and CAT activities were significantly high in bananas, figs, garlic and ginger, based on the fresh and dry weight. Grapes also showed high activities of GSH-PX and CAT but were low in SOD activity. On the other hand, wheat, gourd and lentils were moderate in their content of GSH-PX and CAT but were high in SOD activity. These findings could explain, in part, the variable pattern of antioxidant activity exhibited by the various plants and fruits tested.

SOD activity was reported to be negatively correlated to the content ratio of O_2 and H_2O_2 [12]. Therefore, it was proposed that, in living tissue, the activity of this enzyme play a key role in defense mechanisms against ROS build–up [13]. They reported a sharp increase in SOD activity during early stages of bud swelling, followed by its rapid decline during bud growth. Superoxide dismutase is involved in breaking off bud dormancy by detoxifying the tissue from free radicals [11].

Catalase is one of the most important antioxidant enzymes scavenging the active oxygen species in plant cells [5]. The results of this study showed that CAT activity in all samples was significantly higher than SOD and GSH-PX activities. This finding conforms the results obtained by Abassi *et al.* [11]. These authors found that CAT activity remained relatively high during fruit development. Furthermore, Kotsonis and Mackey, [14] reported that approximately 2% of oxygen consumption during respiration results in H_2O_2 formation. Therefore, it is possible that the increase in CAT activity at these stages could have resulted from an accumulation of H_2O_2 due to a higher rate of respiration.

In addition, enhancement of CAT activity together with decline of SOD activity, suggests a possible build-up of H_2O_2 from an alternative source. Sala *et al.* [15] reported that H_2O_2 is generated during polyamine oxidation and biosynthesis, which are very active during flower and fruit development. Based on these results, CAT activity appears to be linked to the availability of its substrate, H_2O_2.

However, the highest activity of CAT among the two other antioxidant enzymes (SOD and GSH-PX) in this study, suggest that CAT is a key enzyme in active oxygen detoxification during fruit development, which agrees with some other investigators who found that high CAT activity accompanies high growth and respiratory rate and SOD is not the primary enzyme for H_2O_2 formation [16]. Sala and Lafuente [15] have also induced that CAT may be the major antioxidant enzyme involved in plant defense mechanism because it is induced during heating and persists during cold storage.

Catalase and SOD activities were very low in fresh cucumber and grapes, respectively. The decrease in these enzymatic activities suggests that cellular membranes are weakened due to ripening, which causes a significant decrease in CAT, SOD and GSH-PX activities [11].

Ginger (*Zingiber officinale)* exhibited, in this study, a high radical scavenging activity coupled with a high content of total phenolics and SOD activity. The results of Ajith *et al.* [17] concluded that the hepatic-protective effect of aqueous ethanolic extract of ginger against acetaminophen induced acute toxicity is mediated by enhancing the antioxidant status and the beneficial effects of SOD, CAT, GSH-PX, GST and GSH.

Bananas are also mentioned in the Qur'an as one of the fruits of Paradise: Amid thornless lote-trees and banana-trees (with fruits), one above another. And extended shade and water flowing constantly and abundant fruit, neither intercepted nor forbidden.

(Surat al-Waqi'a: 28-33). In this study, banana (*Musa cavendish)* pulp showed high radical scavenging activity, which was coupled with a highest activity of the three antioxidant enzymes (SOD, CAT and GPX). Yet this fruit exhibited a moderate level of total phenolics as compared to grapes or pomegranate. These results complement a study made by Kanazawa and Sakakibara [18] who were able to demonstrate the presence of a strong water soluble antioxidant in the popular commercial banana. It was dopamine, one of the catecholamines, which has a stronger antioxidative property than glutathione but similar to the strongest antioxidants; gallocatechin, gallic acid and ascorbic acid. Banana was shown to contains dopamine at high levels in both the peel and the edible pulp. Perez-Perez and his coworker [19] proved the antioxidant activity of banana by 5 methods and they ascribed this activity to the high concentration of phenolic groups found in all extracts studied. Vijayakumar *et al.* [20] demonstrate that the activities of SOD and CAT were enhanced and the concentration of GSH were increased significantly in rats treated with flavonoids extracted from banana.

In the first Qur`anic verses in Sura al Teen, the medicinal advantages of the fig are discussed-the Quran says "I swear by the Fig and the Olive" (Sura no. 95:verse no 1). According to Abu Darda (Radiallah Anhu) someone presented figs to the prophet Muhammed (Peace be upon him) and he began distributeing it among his followers. He said "Eat it as it cures various diseases".

Six fig varieties differing in color were analyzed by Solomon *et al.* [21] for total polyphenols, total flavonoids, antioxidant capacity and the amount of anthocyanins. They found various concentrations of anthocyanins with a similar profile in all varieties studied. Hydrolysis revealed the presence of cyanidin as the major aglycon (a non-sugar moiety) as the main anthocyanin in all fruits. Another study [22] identified the following phenolics in fig fruits: gallic acid, chlorogenic acid, syringic acid, catechin, epicatechin and rutin. Extracts of darker varieties of figs showed higher contents of phytochemicals compared to the lighter colored varieties. Fruit skins contributed most of the above phytochemicals and of the antioxidant activity, as compared to the fruit pulp.

Garlic: Quran refers its names in Surah Al-Baqarah in the words: and its garlic it is one of the spices. The results of this study show also that garlic *(Allium sativum)* and onion *(Allium cepa)* possess moderate radical scavenging activities, which is accompanied by both moderate phenolic content and moderate antioxidant enzymes when compared with red grapes or pomegranate for example. Garlic extracts reduced the DPPH radical formation (IC_{50} ranging from 1.03 to 6.01 mg/ml) as reported by Bozin *et al.* [23] which matches our results. A recent study by Al-Numair [24] was designed to evaluate the effect of garlic extract on lipid profiles and oxidative stress in male albino rats fed a high cholesterol diet. Garlic extract significantly increased plasma HDL-cholesterol and decreased plasma (LDL-cholesterol and TG) as well as liver TG. Garlic extract significantly increased total antioxidant capacity, SOD and GSH-PX activities and plasma malondialdehyde.

Other study [25] on the antioxidant effect of oils isolated from onion and garlic on nicotine-induced lipid peroxidation in rat tissues found TBARS, conjugated dienes and hydroperoxides concentrations were increased significantly while the activities of CAT, SOD and GSH-PX decreased. Both the garlic oil and onion oil supplementation to nicotine-treated rats increased resistance to lipid peroxidation, increased activities of antioxidant enzymes and increased concentrations of glutathione and they were significantly raised in all tissues studied (liver, lungs and heart).

Ginger (*Zingiber Officinale*) "And they will be given to drink there, a cup (of pure, drink) mixed with Zanjabeel (ginger)" (Sura no. 76:verse no 17). It seems that Arabs, of the past, used to drink two kinds of wine in two forms: One of them was warm and stimulating, which was mixed with ginger; and the other one was cool and narcotic, which was mixed with camphor.

It contains (volatile oil) and substances responsible for the pungent flavor of fresh ginger; these substances are phenolic ketones known as 4-, 6-, 8-, 10- and 12-gingerol which have a high antioxidant activity. Consumption of ginger extract may be proven beneficial in attenuation of atherosclerosis development, since it is associated with reduced macrophage-mediated oxidation of LDL, reduced uptake of oxidized LDL by macrophages, reduced oxidative state of LDL and reduced LDL aggregation [26].

Moreover, they found that ginger extract showed an antioxidant activity comparable with that of butylated hydroxyl toluene (BHT) in inhibiting the lipid peroxidation both at 37°C and at a high temperature of 80°C. Most inhibited was the stage of formation of secondary products of the auto-oxidation of fats. For instance, Ginger used as an antibiotic, then, it is possible that ginger extracts can be used to protect immune-depressed patients [27].

In conclusion, in some of fruits there is a slight indication that catalase activity is highest during the summer, but there is not enough evidence to be at all certain. All food mentioned in the quran has both benifical elements for spirituality and also for the physical components of man.

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