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RESEARCH PAPER

Influence of Processing on Phytochemical Characteristics and *in vitro* antioxidant actvity of *Ficus carica* l. (FIG) products

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Abstract

The present study was undertaken to assess the effect of processing into jam and nectar on phytochemical characteristics, in vitro antioxidant activity viz. reducing power, iron chelating activity, DPPH, ABTS, nitric oxide, superoxide and hydrogen peroxide radical scavenging assays of figure Ficus carica L. fruit. Figure pulp extracts exhibited significantly higher antioxidant activity than fig jam and fig nectar extracts. Total phenolics however to decreased by 10 and 55 %, flavonoids by 98 and 45%, anthocyanins by 79 and 33% and tannins by 83 and 77% in fig jam and fig nectar, respectively, when compared with the fig pulp. However, in vivo studies to determine the antioxidant potential of the fruit need to conducted before it can be recommended as nutritional substitutes.

Keywords: *Ficus carica* L., Processing, jam, nectar, reducing power, iron chelating activity, nitric oxide, superoxide, hydrogen peroxide radical scavenging activity.

At present times, it is believed that the regular consumption of dietary antioxidants may reduce the risk of several serious diseases (Hunter and Fletcher, 2002). Regular consumption of fruits and vegetables has always been associated with health benefits, but their mechanism has become clear only in the recent decades. Fruits and vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals. These phytochemicals impart health benefits beyond basic nutrition (Oomah and Mazza, 2000). Studies have shown that regular consumption of dietary antioxidants lower the risk of several diseases (Wargovich, 2000; Kahkonen *et al.* 1999; Hunter and Fletcher, 2002). The common FIG (*Ficus carica* L.) is a tree native to southwest Asia and the eastern Mediterranean region, belong to botanical family *Moracea*. Fresh fruits have a short, post-harvest life of 7– 10 days, but with a combination of cold storage and a CO_2-enriched atmosphere, the fruit can be stored for up to 2–4 weeks (Sozzi *et al.* 2005). They contain phenolic substances; have a positive effect on human health. Although FIGS are an important fresh fruit as well as a delicious dried fruit and are consumed in most parts of the world, there are only a few reports dealing with the phytochemical composition of this fruit, and there is dearth of information on the effects of processing methods on the *in vitro* antioxidant activity of fig products. Therefore, it is pertinent to have the information on

nutritional composition and influence of processing methods on antioxidant activity of fig products, which may be useful in finding out the technological solutions to preserve their antioxidant activity and improving the bioavailability. So, the present study was conducted to determine the *in vitro* antioxidant activity of fig pulp and its products.

Materials and Methods

Preparation of FIG products

FIG (*Ficus carica* L.) fruit was purchased from local market of Anantapur, Andhra Pradesh (India). Fully mature fruits were selected, washed thoroughly with distilled water and non edible portion of fruits were removed.

FIG *pulp:* Five kg fig fruits were blended in a blender to a smooth pulp. The fruit pulp was kept in sterilized jars and stored in refrigerator for analysis.

FIG *jam*: One kg fig pulp was taken and cooked slowly with gradual addition of one kg sugar and 2.5 g citric acid till the temperature reached 105^0 C and the cooking mass approached 68^0 Brix determined using an Abbe refractometer. The jam was filled in clean dry jars and stored in refrigerator for quality analysis.

FIG *nectar*: One kg fig pulp was taken and heated slowly with gradual addition of 600 g sugar, 2400 ml water and 2.5 g citric acid till the contents mixed properly. The nectar was filled in clean dry bottles and stored in refrigerator for quality analysis.

*Determination of phytochemical constituents***:** Tannins were estimated by Vanillin hydrochloride method given by Price *et al*. (1978), whereas sample preparation for other phytochemicals viz. total phenols, total flavonoids and total anthocyanins was carried out according to Counet and Collin, (2003). Total phenols in the samples were determined following method given by Caboni *et al*. (1997), total flavonoids by Lamaison and Carnat, (1990) and total anthocyanins by Guisti and Wrolstad, 2001 method.

In vitro antioxidant activity

Preparation of extracts: Five gram of the sample was taken in a soxhlet extractor and extracted with 95% ethanol for 72 h. The extract recovered by distillation was used in subsequent experiments.

ABTS radical scavenging activity: ABTS radical cation was produced by reacting ABTS solution (7mM) with 2.4 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 h before use. For the study, different concentrations (50-250µg/ml) of ethanolic extract (0.5ml) were added to 0.3 ml ABTS solution and the final volume was made up with ethanol to make 1 ml. The absorbance was read at 745 nm and the experiment was performed in triplicate (Shirwaikar *et al.* 2004).

DPPH radical scavenging activity: To 1 ml of fruit extract at various concentrations (40-240µg/ml) in methanol, 1 ml DPPH was added. It was then, incubated at 37ºC for 30 minutes. The absorbance of the test mixture was read at 517 nm. Experiment was performed in triplicate and percentage inhibition of DPPH radical was calculated (Sreejayan and Rao, 1996).

Iron chelating activity: The reaction mixture contained 1ml O-phenanthroline, 2ml ferric chloride and 2 ml of ethanolic extract and was incubated at ambient temperature for 10 minutes. The absorbance of the sample was read at 510 nm against blank containing 2 ml of ethanol instead of sample. The experiment was performed in triplicate (Benzie and Strain, 1996).

Reducing Power: Different concentrations of the ethanolic extract (60-240 µg/ml) were mixed with 2.5 ml of phosphate buffer and 2.5 ml of 1% potassium fericyanide in 10 ml test tubes. The mixtures were incubated for 20 min at 50ºC. At the end of the incubation, 2.5ml of TCA was added to the mixtures, followed by centrifuging at 5000 rpm for 10 min. and the upper layer (2.5ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$ and the absorbance was read at 700 nm. Experiment was performed in triplicate (Jayaprakash *et al.* 2001).

Nitric oxide radical scavenging activity: To 1 ml of sample extract in phosphate buffer containing different concentrations (30- 240 µg/ml), 1ml of sodium nitroprusside was added. The mixture was incubated at 25ºC for 150 min followed by addition of 1 ml of griess reagent. Butylated hydroxyl toluene was taken in different volumes to make different concentrations and

run in a similar way. Absorbance was read at 546 nm and the percentage inhibition was calculated (Sreejayan and Rao, 1997).

Superoxide radical scavenging activity: To 3 ml of sample extract in phosphate buffer (to produce concentrations of 30- 200 µg/ml), 1 ml of NBT and 1 ml of NADH were added. The reaction was started by the addition of 100 µl of PMS and the mixture was then, incubated at 25ºC for 5 minutes followed by measurement of absorbance at 560 nm against Butylated hydroxyl toluene as positive control (0.2 mg/ml), which was taken in different volumes (200-1000 µl) to obtain different concentrations and treated in a similar way (Robak and Gryglewski, 1998).

Hydroxyl radical scavenging activity: Varying volumes of extract (50-250 µg/ml) were mixed with 0.02 ml of 100 mM KH_2PO_4- KOH buffer (pH 7.4), 0.2 ml of 0.2 mM $FeCl_3$, 0.1 ml of mM EDTA, 0.1 ml of 10 mM H_2O_2 and 0.2 ml of 2- deoxyribose and mixed thoroughly. It was incubated at room temperature for 60 min. Then, 1 ml of 1%TBA (1g in 10 ml of 0.05 NaOH) and 1 ml of 28%TCA were added. All the tubes were kept in hot water bath for 30 min. BHT was used as a ppositive control for comparison. Absorbance was read at 532nm with reagent blank containing ethanol in place of extract. Decreased absorbance of reaction mixture indicated increased hydroxyl radical scavenging activity (Halliwell *et al.* 1987).

Statistical analysis: The data obtained were analyzed statistically for analysis of variance in a completely randomized design (Sendecor and Cochran, 1994). The data were expressed as the mean ± standard deviation (three replicates) and a probability of ($P \leq 0.05$) was considered to be statistically significant.

Results and Discussion

Phytochemical content of fig pulp and its products is presented in Table 1. Table 1 shows a decrease in all the studied phyto-chemical components in fig jam and nectar samples when compared with fig pulp. The decrease in all the studied phytochemical components can be due to addition of sugar in the products and use of heat in the processing which leads to the various physic-chemical changes in the fruits. Total phenolics were found to decrease by 25 % and 52 % in fig jam and fig nectar, respectively, when compared with the fig pulp. Phenolic compounds are antioxidants and are subjected to oxidation during storage and processing of foods (Titchenal and Dobbs, 2004).

Physical and biological factors such as temperature increase and enzymatic activity might have in the destruction of phenolics. Enzymes such as polyphenol oxidase are responsible for the browning reaction of phenolics but they are normally inactivated during jam preparation due to processing/ cooking at high temperatures. However, there could be losses of bioactive phenolics due to the cooking process that may decrease their functional properties (Kim and Zakour, 2004). The decrease in the phenolic content of fig nectar can be attributed to the oxidation of polyphenols by endogenous polyphenol oxidase and ascorbic acid oxidase (Giovanelli *et al*. 2001 a). The decrease in the flavonoid content of fig jam and fig nectar when compared with fig pulp can be due to the addition of sugar in the processing which did not contribute any flavonoid content to the developed products. As reported by Crozier *et al*. 1995; Price and Rhodes, 1997, decrease is probably due to chemical or thermal degradation. However, fruit nectar processing can increase the flavonoid content as extraction processes can release flavonoids from the rind (Sluis *et al.* 1997) and this explains comparatively lesser decrease in the flavonoid content of fig nectar than fig jam. Total anthocyanins were not detected in fig pulp and its products viz. fig jam and fig nectar. Significant loss of tannins in fig jam and fig nectar than fig pulp was observed due to thermal degradation of tannins. The considerable decrease of tannins in the products can be due to extraction and release of tannins from the cell matrix, due to breakage of bonds with proteins (Rehman and Shah, 2001).

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. The DPPH radical has widely been used in model systems to investigate the scavenging activities of antioxidative compounds. DPPH· is a stable free radical and

accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compounds or extracts with DPPH· in methanol solution. In the presence of hydrogen donors, DPPH· is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by the decrease of the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears (Brand *et al.* 1995). DPPH radical scavenging activity of fig pulp, fig jam and fig nectar extracts at varying concentrations (50– 250 μg/ml) were measured and the results are depicted in Fig 1 (a). All the studied extracts viz. fig pulp, fig jam and fig nectar showed appreciable free radical scavenging activities. Fig pulp had the strongest radical scavenging activity while fig nectar showed the lowest free radical scavenging activity. A dose-response relationship was found in the DPPH radical scavenging activity i.e. the activity increased with an increase in the concentration of extract. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar ($P \leq 0.05$) (Table 2). DPPH scavenging activity of the extracts can be correlated to the presence of flavonoids and phenolic compounds (Khalaf *et al.* 2008.). The phenolic compounds present can act as free radical scavengers by virtue of their hydrogen-donating ability (Shahidi *et al.* 2007).With their high content of phytochemicals such as flavonoids, tannins, stilbenoids, phenolic acids and lignans fruits and fruit products are potentially excellent antioxidant sources. During the processing of fruits to jams, total phenol content is reduced (Amakura *et al.* 2000) resulting in lower antioxidant values in processed fruit products than in fruit pulp. Figure 1(b) represents the ABTS radical scavenging of fig pulp, fig jam and fig nectar. The ABTS radical, generated by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen- donating antioxidants (scavengers of aqueous phase radicals) and of chainbreaking antioxidants (scavengers of lipid peroxyl radicals). All the extracts exhibited ABTS radical scavenging activity in a concentration dependant manner. The scavenging activity was observed in the following order: Fig pulp> fig jam > fig nectar. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar ($P \leq 0.05$) (Table 2). Fruits and vegetables are significant sources of phytochemicals, but processing decreases the phenolic content and hence, affects their potential as radical scavengers. Earlier researches have also reported significant effect on the antioxidant potential of fruits and vegetables by both cooking and storage (Nicoli *et al.* 1999).

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. The iron-dependent decomposition of hydrogen peroxide produces hydroxyl radical, a highly reactive and biologically toxic species, via the Fenton reaction. This reductive cleavage of H_2O_2 is considered as the main source of hydroxyl radical in vivo, and is a major mechanism of biological damage (Mao *et al.* 1996). Fig 1 (c) shows hydroxyl radical scavenging activity in fig pulp, fig jam and fig nectar. The per cent scavenging activity with respect to all the extracts increased as concentration of extracts increased from 50- 250 μg/ml. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar ($P \leq 0.05$) (Table 2). This can be correlated to the presence of total phenols in the extract. The hydroxyl radical scavenging activity is attributed to the radical scavengers like flavonoids, phenols, vitamin c and β carotene present in fruits (Elmastas *et al.* 2006).

Antioxidants act by scavenging the NO radicals (Chandha and Dave, 2009). The NO scavenging activity in fig pulp was found to be greater than fig jam and fig nectar [Figure 1 (d)]. The per cent scavenging activity with respect to all the extracts increased as concentration of extracts increased from 50- 250 μg/ml. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar ($P \leq 0.05$) (Table 2). Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds (Sonawane *et al.* 2010).

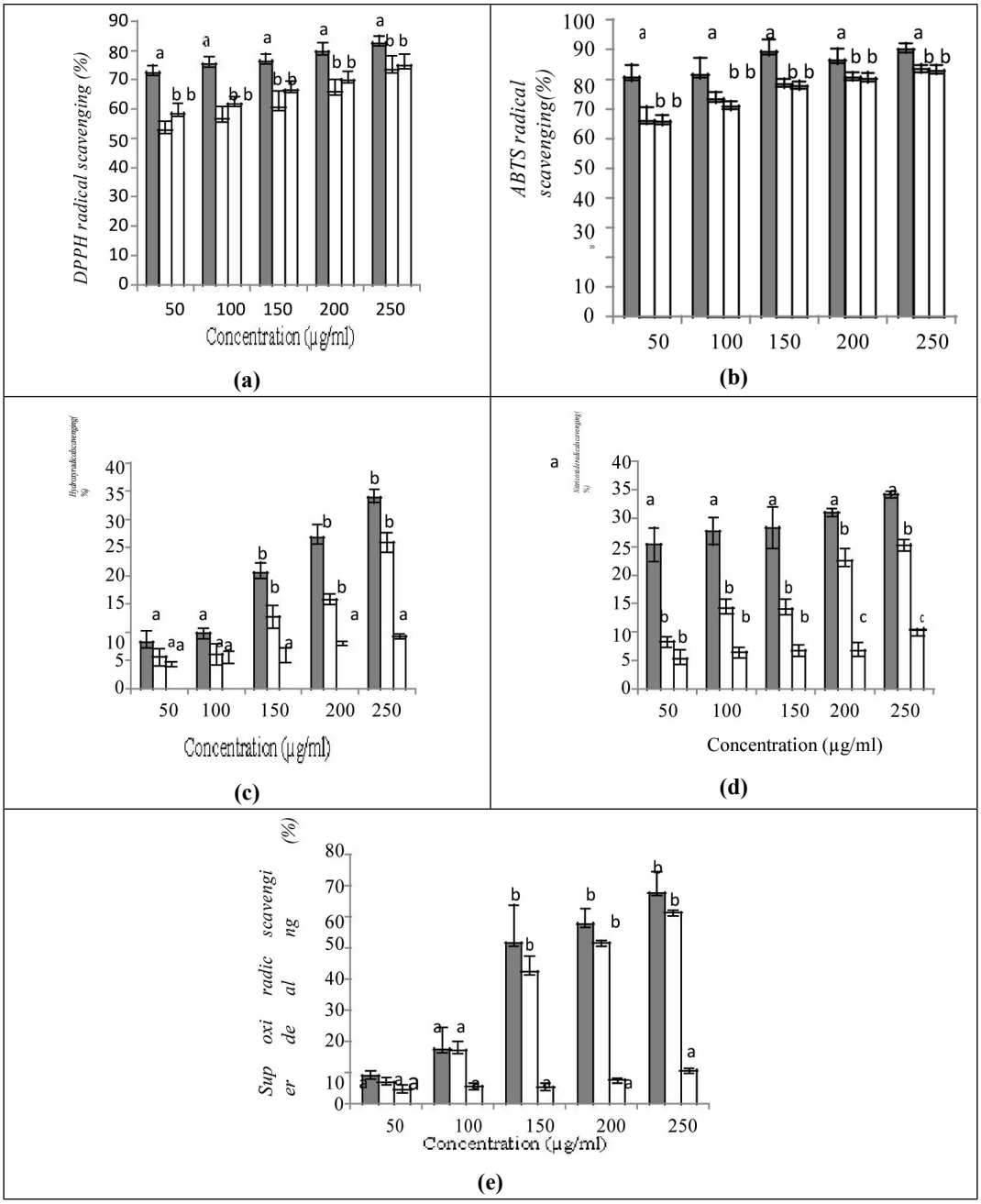

Fig. 1: Radical scavenging activities of fig pulp, fig jam and fig nectar extracts (a) DPPH (2,2-diphenyl- 1-picrylhydrazyl) radical scavenging activity (b) ABTS (2,2'- azinobis 3- ethyl benzothiazoline-6- sulphonic acid diammonium salt) radical scavenging activity (c) Hydroxyl radical scavenging activity (d) Nitric oxide radical scavenging activity (e) Super oxide radical scavenging activity

*Values are mean ± SD of 3 observations

Bars marked with same letters are not significantly different (P<0.05)

Table 1: Influence of processing on the phytochemical composition of fig pulp, jam and nectar

Sample/ Parameters	Fig pulp	Fig jam	Fig nectar
Total phenolics (mg %)	161.2±0.82	133.3± 0.20 (25)	77.8± 0.72 (52)
Total flavonoids (mg %)	4.4 ± 0.93	0.4± 1.10 (98)	2.0± 0.61 (45)
Total anthocyanins	0.5±0.92	0.2±0.71 (79)	0.4±0.73 (33)
Tannins (mg %)	100.6 ± 0.43	16.6±1.02 (83)	22.5± 0.72 (77)

Values are mean ± SEM of 3 observations

Figures in the parenthesis indicate % decrease over the fruit pulp values

Table 2: Significant differences between antioxidant activities of fig pulp and its products

Fig Pulp and its Products	DPPH radical scavenging activity	ABTS radical scavenging activity	Hydroxy radical scavenging activity	Nitric oxide radical scavenging activity	Super oxide radical scavenging activity
Fig Pulp vs Fig Jam	*	*	ns	*	ns
Fig Pulp vs fig Nectar	*	*	*	*	*
Fig Jam vs Fig Nectar	ns	ns	ns	*	*

ns not significant

*** Significantly different at 5% level**

Superoxide anion is a weak oxidant; still it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Chandha and Dave, 2009). The SO scavenging activity was found to be very high in both the extracts. SO scavenging activity is associated to total flavanoids which is higher in the methanolic extract (Chen and Yen, 2006; Chandha and Dave, 2009). Superoxide radical scavenging activity of fig pulp, fig jam and fig nectar is presented in Fig. 1 (e). Figure pulp exhibited higher scavenging activity than its processed counterparts, owing to comparatively higher per cent of phenolics, flavonoids, tannins and vitamin C. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar (P≤0.05) (Table 2). Flavonoids inhibit lipid peroxidation *in vitro* at the initiation stage by acting as scavengers of superoxide anions and hydroxyl radicals (Afanas *et al.* 1989).

The antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power (Oyaizu, 1986). Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. Among fig pulp, fig jam and fig nectar, fig pulp had the highest reducing power (Fig. 1). At concentrations of 50-250 µg/ml, reducing power of the extracts followed the order fig pulp > fig jam> fig nectar. Fig pulp exhibited reducing power in the range from 0.45±0.03 (%) to 0.83±0.05 (%), whereas fig jam and fig pulp from 0.26±0.01 (%) to 0.53±0.03 (%) and 0.32 ± 0.03 (%) to 0.48±0.05 (%), respectively. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar (P≤0.05). The reducing power is mainly correlated to the presence of reductones like ascorbic acid (Li *et al.* 2008).The reducing power of the extracts of fig pulp, fig jam and fig nectar increased with increasing concentrations of the extracts. Ferrous ion, commonly found in food

systems, is well known as an effective pro-oxidant (Chandha and Dave, 2009). The purpose of the test of ferrous ion chelating activity was to determine the capacity of fig pulp, fig jam and fig nectar to bind the ferrous ion catalyzing oxidation. Ferrozine can quantitatively form complexes with Fe2+. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex decreases. A measure of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator (Yamaguchi *et al.* 2000). In this assay, the extracts of fig pulp, fig jam and fig nectar interfered with the formation of a ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ions before ferrozine. Fig pulp exhibited highest ferrous ion chelating activity (0.13± 0.03) followed by fig jam (0.10±0.04) and fig nectar (0.03±0.01). A dose dependent relationship was observed in the ferrous ion chelating activity of the fig pulp, fig jam and fig nectar. ANOVA results revealed significant differences in the free radical scavenging activity among fig pulp, fig jam and fig nectar ($P \leq 0.05$).

Conclusions

Processing of fig fruit pulp into jam and nectar resulted in a significant decrease in phytochemical composition of the products. Among the extracts analyzed, fig pulp exhibited the highest antioxidant activity followed by fig jam and fig nectar, as analyzed by different methods. The consumption of these fruits and fruit may play a role in preventing human disease in which free radicals are involved, such as cancer, cardiovascular diseases and aging. However, further investigations on individual components, their in *vivo* antioxidant activity, and the different antioxidant mechanisms are warranted.

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References

Afanas'ev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A. and Potapovitch, A.I. 1989. Chelating and free radical scavenging mechanisms and inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol* **38**: 1763-1769.

Amakura, Y., Umino, Y., Tsuji, S. and Tonogai, Y. 2000. Influence of jam processing on the radical scavenging activity and phenolic content in berries. *J Agric Food Chem* **48**: 6292-6297

Benzie, IFF and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant" power: the FRAP assay. *Anal Biochem* **239**: 70-76.

Brand-Williams, W., Cuvelier, M.E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* **28**: 25–30

Caboni, E., Tonelli, M.G., Lauri, P., Lacovacci, P., Kevers, C., Damiano, C. and Gaspar, T. 1997. Biochemical aspects of almond microcuttings related to in vitro rooting ability. *Biol plant* **39**: 91-97.

Chandha, S. and Dave, R. 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research* **3**: 981-996.

Chen, H., Yen, G. 2006. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. *Journal of Agricultural and Food Chemistry* **2**: 686-694.

Counet, C. and Collins, S. 2003. Effect of the number of flavonol units on the antioxidant activity of procyanidin fractions isolated from chocolate. *Journal of Agricultural Food Chemistry* **51**: 6816-6822.

Crozier, A., Lean, M.E.J., Mc Donald, M.S. and Black, C. 1995. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *Journal of Agricultural Food Chemistry* **42**: 281-285.

Elmastas, M., Ibao, K. and Gulcin, I. 2006. Radical Scavenging Activity and Antioxidant Capacity of Bay Leaf Extracts. *Journal of the Iranian Chemical Society* **3**: 258-266.

Giovanelli, G., Lavelli, V., Peri, C., Pagliarini, E., Zanoni, B. and Spingno 2001. The antioxidant activity of tomato. Effects of processing technologies on oxidative and heat damage. *Acta. Hort.* **542**: 217-220.

Guisti, M.M. and Worlstad, R.E. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Current protocols in Food analytical chemistry, Worlstad RE and Schwartz SJ (eds.). Wiley------- New York, pp F1.21- F1.2.2.13

Halliwell, B., Gutteridge, J.M. and Aruoma, O.I. 1987. The deoxyribose method: a simple'rest tube' assay for determination of rate constants for reaction of hydroxyl radicals. *Anal. Biochem*. **165**: 215-219.

Hunter, K.J., Fletcher, J.M. 2002. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetable. *Innov Food Sci Emerg Technol* **3**: 399–406 doi:10.1016/S1466-8564(02) 00048-6.

Jayaprakash, G.K., Singh, R.P. and Sakariah, K.K. 2001. Antioxidant activity of grape seed extracts on peroxidation models in-vitro. *J. Agric Food Chem*. **55**: 1018-1022.

Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J-P., Pihlaja, K., Kujala, T.S. and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 473954–3962 doi: 10.1021/jf990146l

Khalaf, N., Ashok, K. and Shakya, A. 2008. Antioxidant Activity of Some Common Plants. *Turk J Bio* **32**: 51-55.

Kim, D.O. and Padilla Zakour, O.I. 2004. Jam processing effect on phenolics and antioxidant capacity in anthocyanins-rich fruits: Cherry, Plum and Raspberry. *Journal of food scienc*e **69**: 395-400.

Lamaison, J.L. and Carnat, J. 1990. Teneur en principaux flavonodes does fleurs et des feuiltes de crtaegus monogyna Jacq.et.de Crataegusleavigata (Poret) DC. (*Rosaceae*). *Acta Pharm. Acta Pharm. Acta Helv* **65**: 315-320.

Li, H., Li, Y. and Wang, X. 2008. Comparative Study of Antioxidant Activity of Grape (*Vitis vinifera*) Seed Powder Assessed by Different Methods. *Journal of Food and Drug Analysis* **16**: 1-7.

Nicoli, M.C., Anese, M. and Parpinel, M. 1999. Influence of processing on the antioxidant properties of fruits and vegetables. *Trends in Food Science and Technology* **10**: 94-100.

Oomah, B.D., Mazza, G. 2000. Functional foods. In: Francis FJ (ed) The Wiley encyclopedia of science & technology, 2nd edn. Wiley, New York, pp 1176–1182

Oyaizu, M. 1986. Studies on product of browning reaction produced from glucose amine. *Jap J Nutr* **44**: 307– 315

Price, M.L., Van Scoyoc, S. and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry* **26**: 1214–1218.

Robak, J. and Gryglewski, R.J. 1988. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmac* **37**: 837-841.

Sendecor, W. and Cochran, W.G. 1994. Statistical methods. Sixth edn., Oxford and IBH Publication, New Delhi p 297.

Shahidi, F., Alasalvar, C. and Liyana-Pathirana, C.M. 2007. Antioxidant phytochemicals in hazelnut kernel (*Corylus avellana* L.) and hazelnut by product. *Journal of Agricultural and Food Chemistry* **55**: 1212–1220.

Shirwaikar, A., Rajendran, K. and Kumar, C.D. 2004. In vitro antioxidant studies of *Annona squamosa* Linn leaves. *Ind J Exp Biol* **42**: 803-807.

Sonawane, I., Dhasade, V., Nirmal, S. 2010. Antioxidant effect of Tephrosia Purpurea L. roots. *International Journal of Pharmaceutical Sciences and Research* **1**: 57-60.

Sozzi, G.O., Abrajan-Villasenor, M.A., Trinchero, G.D. and Fraschina, A.A. 2005. Postharvest response of 'Brown Turkey' figs (*Ficus carica* L.) to the inhibition of ethylene perception. *Journal of the Science of Food and Agriculture* **85**: 2503–2508.

Sreejayan, and Rao, M.N. 1997. Nitric oxide scavenging by curcuminoids. *J. Pharm. Pharmacol* **49**: 105-107.

Sreejayan, N. and Rao, M.N. 1996. Free radical scavenging activity of curcuminoids. *Arzneimittelforschung* **46**(2): 169-171.

Titchenal, C.A. and Dobbs, J. 2004. Nutritional value of vegetables, In: Handbook of vegetable preservation and processing. Hui YH, Ghazala S, Graham DM, Murell KD and Nip W (eds.), Marcel Dekker, New York, pp 23- 37.

van der Sluis, A.A., Dekker, M., de Jager, A. and Jongen, W.M.F. 2001. Activity and concentration of phenolic antioxidants in apple: effect of cultivar, harvest year and storage conditions. *Journal of Agricultural and Food Chemistry* **49**: 3606–3613.

Wargovich, M.J. 2000. Anticancer properties of fruits and vegetables. *Hortic Sci* **35**: 573–575

Yamaguchi, F., Ariga, T., Yoshimira, Y., Nakazawa, H. 2000. Antioxidant and anti-glycation of carcinol from *Garcinia indica* fruit rind. *J Agric Food Chem* **48**: 180–185 doi:10.1021/jf990845y