

## EFFECT OF FRESH WHEATGRASS JUICE ON PLASMA ANTIOXIDANT STATUS OF GERIATRIC POPULATION

SHARMA, B., PAREKH, T.<sup>?</sup> DAVE, N., PATEL, V. AND REMA, S.

Laboratory of Food Science and Nutrition, Department of Home Science, Sardar Patel University, Vallabh Vidyanagar-388120, E. mail: [tarak577@gmail.com](mailto:tarak577@gmail.com)

Received: August 4, 2008; Revised: October 15, 2008; Accepted: 28, 2008

**Abstract:** *Wheat grass is considered to be a nutritional powerhouse. It acts as a natural cleanser of the body. The present study was undertaken with the broad objective of assessing plasma antioxidant levels of geriatric population after supplementation of wheat grass juice for one month. Fresh wheatgrass was analyzed for total phenol, flavonoids and antioxidant capacity. 18 subjects were selected for the study. All experimental subjects were fed with fresh wheat grass juice daily early in the morning for 30 days. It was observed that 30 days supplementation of juice significantly ( $P < 0.01$ ) increased the hemoglobin, glutathione, ascorbic acid and vitamins A, E, TAC (mg trolox equivalent per 100 gm). The phenol content of water and methanolic extract was found to increase tremendously. The water extract showed significantly higher ( $p < 0.01$ ) TAC compared with the methanolic extract. It is concluded from the study that wheat grass juice provides genuine therapeutic advantage in the body and can be used as a nutritional supplement for therapeutic implication in the treatment of various diseases.*

**Key words:** Wheatgrass, Antioxidant, Plasma glutathione, Geriatric population

### INTRODUCTION

Wheat grass juice is an extract squeezed from mature sprouts of wheat seeds (*Triticum aestivum*). It is rich in vitamin A, C and E and an excellent source of essential B vitamins, which are necessary for normal brain and bone development and general metabolism of body. Wheat grass juice contains 70 % chlorophyll. It has dilating effect on the blood vessels, increases the iron content in the blood and helps in rebuilding and detoxifying the body [1].

The use of wheat grass juice for therapeutic purposes was developed and popularized by Dr. Ann Wigmore [1]. Although proponents of wheat grass juice was recommended for the last three decades as a treatment of various ailments, including chronic inflammatory conditions and malignancies, to date no clinical trials exist. The juice is believed to possess therapeutic qualities only when fresh, therefore, should be consumed immediately after extraction on an empty stomach. The therapeutic qualities of juice

have been attributed to its rich nutritional content like chlorophyll, vitamins (A, C and E), bio-flavonoids, minerals (iron, calcium and magnesium), and 17 amino acids, 8 of which are essential [2]. Several studies have assessed an anti-mutagenic property of wheat grass juice. In 1979, Lai et al. demonstrated an antimutagenic property of wheat grass extract in the Ames test system and attributed it to chlorophyll [3]. In 1992, Peryt showed its anti-mutagenic property against cyclophosphamide and ethidium bromide, which was related to flavonoids, particularly apigenin [4]. It is an excellent antioxidant and plays a role in the body's defense system against "reactive oxygen species" (ROS), which are the harmful by products generated during the cell aerobic respiration [5].

Looking at the importance of wheat grass juice, the present study was carried out to observe the effect of feeding of fresh wheat grass juice on blood antioxidant profile of geriatric people. Along with this, the phenol, flavonoids and antioxidant capacity of fresh wheat grass was also evaluated.

## MATERIALS AND METHODS

**Selection of subjects:** 18 geriatrics (9 male and 9 female) in the age group of 50-65 years were selected from local community of vallabh vidyanagar for the study. The subjects were chosen on the basis of their willingness to co-operate and they were free from any apparent complications. Their anthropometric measurements were taken to confirm that all the subjects were having normal body mass index (BMI).

**Preparation of wheatgrass juice and feeding the subjects:** Wheat grass was procured from a local Nursery farm and washed with potable water. 100 ml of water was taken and from that a small amount was used for crushing 30 gms of wheatgrass in a grinder. Remaining water was mixed with the wheatgrass pulp. It was then filtered through a muslin cloth by squeezing. This filtered extract was fed to the subjects (30 gms wheat grass/100 ml water/person/day for 30 days). The juice was extracted every day freshly and was fed to the subjects early in the morning (6:30 to 7:00 am) for 30 days. The subjects were asked to follow their normal routine dietary pattern of breakfast, lunch and dinner etc.

**(A) Collection and analysis of blood sample:** After an overnight fasting (approximate 12 hours), 5 ml of venous fasting blood sample was drawn from each subject by a pathologist. The blood was collected in a clean dry tube containing dried ethylene diamine tetra acetic acid (EDTA). The blood samples were collected on 0 day (before starting the experiment) and on the 30<sup>th</sup> day (at the end of experiment).

**Hemoglobin estimation:** Hemoglobin content of blood was estimated by the oxy-HB method of Evently and Malloy [7]. 0.02 ml blood was transferred to 8 ml of 1 % ammonia solution, mixed well and then colour intensity was measured at 540 nm.

**Glutathione (GSH):** Glutathione was estimated from the whole blood by the method given by Ellman [8]. 0.4 ml of blood sample was mixed with 0.4 ml of 5 % TCA in plastic vials. This was mixed in the cyclo-mixture and followed by centrifugation at 2000 rpm for 10 minutes to obtain a protein free supernatant. After centrifugation 0.01 ml of supernatant was taken in to plasma tube and 1.99 ml of phosphate buffer (pH-8) and 0.1 ml of DTNB solution were added. This was mixed in cyclo mixture and allowed to stand for 10 minutes at room temperature. The colour developed was read at 412 nm in a double beam UV

visible spectrophotometer (Hitachi 220S, Japan). The standard series was prepared from working reagent ranging from 2 to 10 µg. The volume was made up to 1 ml with phosphate buffer (pH-8) and treated in the same way as sample. Blank was prepared using 1 ml of phosphate buffer (pH-8) and treated in the same way as sample.

**Ascorbic Acid (Vitamin C):** Vitamin C was estimated from whole blood by the method given by Roe and Kuther [9]. Exactly 0.4 ml of blood was mixed with 0.4 ml of chilled 10% trichloro acetic acid (TCA) in the test tube. The content was centrifuged at 5000 rpm for 10 minutes and from this 0.2 ml of supernatant was taken in a plasma tube and volume was made up to 0.5 ml with 5% TCA. 0.1 ml of DNPH-thiourea-CuSO<sub>4</sub> solution (DTC solution) was added. This mixture was incubated at 37 °C for 3 hrs. 0.75 ml of 65 % H<sub>2</sub>SO<sub>4</sub> was added, mixed and allowed to stand at room temperature for 30 minutes. Absorbance was determined at 520 nm in the spectrophotometer. Standards ranging from 2 to 8 µg were prepared. 0.5 ml of 5 % TCA was added and analysed in the same way as sample. Blank consisted of 0.5 ml of 5 % TCA which was then treated in the same way as sample.

**(B) Plasma analysis:** Plasma was obtained by centrifugation of whole blood at 8000 rpm for 10 minutes. The supernatant was collected as plasma.

**Total Antioxidant capacity (TAC) by FRAP method:** The procedure, described by Benzie and Strain [10], was used to evaluate the TAC of plasma. 20 µl of plasma was taken in a test tube and volume was made up to 300 µl with distilled water. 1.8 ml of FRAP reagent was added and allowed to incubate at 37 °C for 10 minute. The coloured complex was measured at 593 nm using double beam U.V. spectrophotometer. Standard series was prepared using known concentration of ascorbic acid (ASA) (1-4 µg), trolox (1-4 µg) and gallic acid (0.2-0.8 µg).

**Vitamin A:** Plasma vitamin A was estimated by the method given by Neeld and Pearson [11]. 0.5 ml of plasma was mixed with 0.5 ml of 1 % pyrogallol solution in a centrifuge tube mixed and incubated at 70 °C for 2 minutes in water bath. 0.1 ml of saturated KOH was added and incubated for 30 minutes at 70 °C in water bath. Sample was cooled in ice and 1 ml of distilled water and 4 ml of chilled hexane were added. This was mixed vigorously for 2 minutes in a cyclo mixer and was followed by centrifugation for

5 minutes at 1500 rpm. The upper layer was carefully taken in dry test tube and known amount was evaporated to dryness at 40 °C in a vacuum dryer.

After vacuum drying, 20 µl of chloroform was added. 1 ml of chloroform- Trifluoro acetic acid (chloroform-TFA reagent) (2:1) reagent was added. Colour intensity was reads at 620 nm in a spectrophotometer within 30 seconds. Standards were prepared ranging from 0.4 µg to 2 µg from the working solution of trans-retinol palmitate and treated as sample. Chloroform was used as blank.

**Vitamin E:** Plasma vitamin E was estimated by modified method given by Desai [12]. 0.5 ml of plasma was mixed with 0.5 ml of 1% pyrogallol solution in a tube and incubated at 70°C for 2 minutes in water bath. 0.1 ml of saturated KOH was added and incubated for 30 minutes at 70°C in water bath. Sample was cooled in ice and 1 ml of distilled water and 4 ml of chilled hexane was added. Mixer was mixed vigorously for 2 minutes in a cyclo mixture. This was followed by centrifugation at 1500 rpm for 5 minutes. The upper layer was carefully taken in dry test tube and known amount was evaporated to dryness at 40°C in a vacuum dryer. After vacuum drying, residue was dissolved in 1 ml of ethanol. 0.2 ml of 0.2 % bathophenanthraline reagent was added and mixed thoroughly. To this 0.2 ml of FeCl<sub>3</sub> reagent was added and mixed well using cyclo mixture. Exactly after one minute 0.2 ml of orthophosphoric reagent was added and colour intensity was read at 536 nm in a spectrophotometer. The standards were prepared ranging from 2 to 8 µg (i.e. 0.1 to 0.4 ml) from working solution of α – tocophoral acetate. The volume was made up to 1 ml with ethanol and treated in the same way as sample. 1 ml of ethanol was used as blank and treated in the same way as sample.

### (C) Wheatgrass analysis:

**Ascorbic acid (vitamin C):** Vitamin C from wheatgrass was determined by using titrimetric method of Rangana [13]. 5 gm of sample was taken for extraction, and then 25 ml of 3 % MPA was added, treated with a pinch of charcoal, and centrifuged for 10 minutes at 8000 rpm. The filtrate was filtered with whatman no.1 filter paper; volume was made upto 50 ml with 3% MPA. 2 ml aliquot was taken in a conical flask and volume was made 10 ml with 3 % MPA. Titrated against standard dye (2,6 dichlorophenol indophenol) till a light pink colour appeared that persisted for 30 seconds.

**Sample preparation for phenol, flavonoids and total antioxidant capacity:** 5 gm of fresh wheatgrass sample was weighed and ground in a mortar and pestle after adding 25 ml of distilled water (for water extract) and 20 ml methanol (for methanol extract). The homogenate was refluxed for two hours for water extract and for one hour for methanol extract. The homogenate was then centrifuged at 6000 rpm for 10 minutes. The supernatant was recovered and used as sample.

**Total phenolic compound:** Total phenolic compounds were estimated according to the method described by Schwarz [14]. 0.05 ml aliquot was taken in a test tube and volume was made up to 1 ml with distilled water. To this 1 ml each of folin-ciocalteau reagent diluted with water (1:2) and 35 % Na<sub>2</sub>CO<sub>3</sub> were added. The contents were incubated for 10 min at room temperature. 2 ml of distilled water was added and intensity of blue colour was recorded at 620 nm, in UV spectrophotometer. Gallic acid of known concentration (5-20 µg) was used as standard.

**Flavonoid:** Flavonoid content was estimated by the method described by Zhishen et al [15]. 0.2 ml of sample was taken and volume was made up to 1 ml of 20 % HCL and 0.5 ml of formaldehyde solution was added to it. It was kept at room temperature for 24 hours. The content was centrifuged at 3000 rpm for 10 minutes. 1 ml of supernatant was treated as same way as for total phenol estimation. Standard and blank were treated as they were total phenol estimation.

**Total antioxidant activity by 1, 1-Diphenyl 2-picryl Hydrazyl (DPPH) method:** The antioxidant activity was determined by the ability of extract to scavenge DPPH radicals as described by Mccue and Shetty [16]. 0.2 ml aliquot of fresh wheatgrass extract was taken and volume was made up to 1 ml with methanol. Then 3 ml of DPPH reagent was added. The content was mixed properly. It was incubated at 37 °C for 20 minutes. After incubation the absorbance was measured at 517 nm. In 1 ml methanol, 3 ml DPPH was added and was used as control. For standard, known concentration of Ascorbic acid (10-40 µg), gallic acid (1-4 µg) and trolox (10-40 µg) were taken. Methanol was used as blank.

**Total antioxidant capacity by FRAP assay:** The FRAP (Ferric Reducing Antioxidant Power) method [10] was used to evaluate the TAC of wheat grass juice. 0.1 ml of fresh extract was taken in a test

tube and volume was made up to 300 µl with distilled water. 1.8 ml of FRAP reagent was added and allowed to incubate at 37 °C for 10 minute. The coloured complex was measured at 593 nm using double beam U.V. spectrophotometer. For standard, known concentration of ascorbic acid (10-40 µg), gallic acid (1-4 µg) and trolox (10-40 µg) were taken. For blank, 300 µl of distilled water was taken and 1.8 ml FRAP reagent was added.

**Statistical analysis:** The results obtained were analyzed using SPSS for windows 10.0. Differences between variables were tested for significance by using one-way analysis of variance (Duncan).

## RESULTS

**Haemoglobin:** Table 1 depicts the mean value of hemoglobin, whole blood glutathione, and ascorbic acid levels of subjects before and after wheat grass juice supplementation. The hemoglobin concentration before supplementation was 9.35 gm % which significantly increased ( $p < 0.01$ ) to 12.98 gm % after fresh wheat grass juice supplementation. The percentage increase was found to be 38.82 %. Chlorophyll makes up > 70 % of the solid content of wheat grass juice. Both chlorophyll and hemoglobin share a similar atomic structure. The only difference in the two molecules is that of the metallic atom element. Hemoglobin consists of iron, while in chlorophyll the metallic atom is magnesium.

**Glutathione:** Table 1 depicts the mean value of glutathione. Glutathione is an important non protein thiol, which in conjunction with glutathione peroxidase and glutathione-s-transferase plays a central role in maintaining the integrity of the cells when challenge by different toxic agents. The liver plays a major role in homeostasis of glutathione, it is used by glutathione peroxidase as a substrate to catalyze the reduction of organic hydroperoxides and hydrogen peroxides. In normal metabolism glutathione plays an important role in detoxification. Therefore in the present study it was analyzed in blood before and after supplementation of fresh wheatgrass juice. The mean value of glutathione before feeding was 42.51 mg/g and after supplementation for one month it increased significantly ( $P < 0.01$ ) to 76.84 mg %.

**Plasma ascorbic acid:** Table 1 depicts the mean value of plasma ascorbic acid. The ascorbic acid concentration before supplementation was 8.19 mg

% which significantly ( $P < 0.01$ ) increased to 13.75 mg % after fresh wheatgrass juice supplementation. The percentage increase was found to be 67.88 %.

**Plasma vitamin A:** Table 1 depicts the mean value of plasma vitamin A. Vitamin A has many physiological functions like normal vision, growth and development and healthy skin. Beta carotene supplementation may prevent cardiovascular disease and cancer because of its antioxidant effects [17]. The initial (before feeding fresh wheatgrass juice) plasma vitamin A level was 75.16 µg % which significantly increased ( $P < 0.01$ ) to 171.45 µg % after wheatgrass supplementation. The percentage increase was found to be 121.11 %.

**Plasma vitamin E:** Table 1 depicts the mean value of plasma vitamin E. vitamin E is a lipid soluble antioxidant and scavenge free radicals and may prevent oxidative damage to lipid membrane and low-density lipoprotein. Vitamin E is also needed in immune function and enhances cell-mediated immunity. Vitamin E deficiency results in fat malabsorption, cystic fibrosis, liver disease, muscles weakness and hemolysis [17].

The initial (before feeding wheatgrass juice) plasma vitamin E level was 0.85 mg % and final (after feeding fresh wheatgrass juice) level was 1.94 mg %. These values were significantly different from each other ( $P < 0.01$ ). After wheatgrass supplementation the percentage increase was found to be 128.23 %.

**Table 1:** Different subjects were fed wheatgrass juice. Values are mean  $\pm$  S.D of seven observations; a significantly different from b ( $P < 0.01$ ); c significantly different from d ( $P < 0.01$ ); e significantly different from f ( $P < 0.01$ )

|                         | Initial level (0 day)         | Final level (30 <sup>th</sup> day) |
|-------------------------|-------------------------------|------------------------------------|
| Hemoglobin (gm %)       | 9.35 <sup>a</sup> $\pm$ 1.16  | 12.98 <sup>b</sup> $\pm$ 1.63      |
| Glutathione (mg %)      | 42.51 <sup>c</sup> $\pm$ 2.69 | 76.84 <sup>d</sup> $\pm$ 9.05      |
| Ascorbic acid (mg %)    | 8.19 <sup>e</sup> $\pm$ 1.60  | 13.75 <sup>f</sup> $\pm$ 2.65      |
| Plasma Vitamin A (µg %) | 75.16 <sup>a</sup> $\pm$ 4.36 | 171.45 <sup>b</sup> $\pm$ 9.67     |
| Plasma Vitamin E (mg %) | 0.85 <sup>c</sup> $\pm$ 0.26  | 1.94 <sup>d</sup> $\pm$ 0.54       |

**Table 2:** Plasma Antioxidant activity of subjects fed fresh wheatgrass juice by FRAP assay. Values are mean  $\pm$  S.D of seven observations; a significantly different from b ( $P < 0.01$ ); c significantly different from d ( $P < 0.01$ ); e significantly different from f ( $P < 0.01$ ).

|               | mg Ascorbic acid<br>Equivalent<br>per 100 gm | mg Trolox<br>equivalent<br>per 100 gm | mg Gallic acid<br>Equivalent<br>per 100 gm |
|---------------|----------------------------------------------|---------------------------------------|--------------------------------------------|
| Initial level | 19.55 <sup>a</sup> $\pm$ 7.2                 | 27.87 <sup>c</sup> $\pm$ 3.3          | 4.61 <sup>e</sup> $\pm$ 0.98               |
| Final level   | 40.31 <sup>b</sup> $\pm$ 8.3                 | 57.13 <sup>d</sup> $\pm$ 3.33         | 11.55 <sup>f</sup> $\pm$ 2.37              |

**Table 3:** Total Phenol and flavonoid content of the fresh wheatgrass. Values are mean  $\pm$  S.D of seven observations; a significantly different from b (Pd<0.01 ); c significantly different from d (Pd<0.01 )

|                  | Phenol (mg %)                  | Flavonoid (mg %)               |
|------------------|--------------------------------|--------------------------------|
| Water extract    | 345.95 <sup>a</sup> $\pm$ 4.57 | 160.85 <sup>c</sup> $\pm$ 6.46 |
| Methanol extract | 172.59 <sup>b</sup> $\pm$ 5.31 | 169.59 <sup>d</sup> $\pm$ 3.36 |

**Table 4:** Antioxidant activity of fresh wheat grass (by FRAP assay). Values are mean  $\pm$  S.D of seven observations; a significantly different from b (Pd<0.01 ); c significantly different from d (Pd<0.01 ); e significantly different from f (Pd<0.01 )

|                    | mg ascorbic acid equivalent per 100 gm | mg trolox equivalent per 100 gm | mg gallic acid equivalent per 100 gm |
|--------------------|----------------------------------------|---------------------------------|--------------------------------------|
| Water extract      | 446.71 <sup>a</sup> $\pm$ 32.84        | 345.92 <sup>c</sup> $\pm$ 19.22 | 117.03 <sup>e</sup> $\pm$ 28.87      |
| Methanolic extract | 354.16 <sup>b</sup> $\pm$ 8.17         | 266.27 <sup>d</sup> $\pm$ 6.63  | 91.50 <sup>f</sup> $\pm$ 4.47        |

**Table 5:** Antioxidant activity of fresh wheat grass (by DPPH assay) .Values are mean  $\pm$  S.D of seven observations; a significantly different from b (Pd<0.01)

|                    | Antioxidant capacity ( % inhibition ) |
|--------------------|---------------------------------------|
| Water extract      | 43.89 <sup>a</sup> $\pm$ 0.56         |
| Methanolic extract | 36.09 <sup>b</sup> $\pm$ 2.16         |

### Plasma total antioxidant capacity by FRAP

**assay:** Table 2 depicts the mean value of plasma TAC. Antioxidant defense system in our body protects against radicals produced during different metabolic processes in our body. In the present study TAC was estimated in response to wheat grass supplementation. Before feeding the fresh wheatgrass juice the plasma TAC equivalent to ascorbic acid, Trolox and Gallic Acid was 19.55, 27.83 and 4.61 mg/100gm respectively, which significantly increased to 40.31, 57.13 and 11.55 mg/100gm respectively. After fresh wheatgrass juice supplementation, the percentage increase equivalent of ascorbic acid, trolox and gallic acid was found to be 106.18 %, 104.98 % and 149.56 %, respectively. The ascorbic acid content of fresh wheatgrass was found to be 28.87 mg %.

**Fresh wheatgrass analysis:** The mean values of phenol and flavonoid content of fresh wheatgrass is presented in table 3. The phenol content of water and methanolic extract was found to be 345.95 mg % and 172.59 mg %, respectively. The water extracts showed significantly higher (pd<0.01) phenolic content as compared to the methanolic extract. The total phenolic content of raw spinach as reported by Gitanjali et al. [18] and Vinson et al. [19] were 115.25 mg % and 1.7  $\mu$ mol/g respectively. The total phenolic

content of raw spring onion was 948 mg/kg as reported by karadeniz et al. [20]. The phenolic content of raw cabbage reported by Chun et al [21] was 97.8 mg %. The flavonoid content of water and methanolic extract was found to be 160.85 mg % and 169.85 mg %, respectively. The methanolic extract showed significantly higher (Pd<0.01) flavonoid content as compared to water extract.

**FRAP assay:** The mean TAC equivalent of ascorbic acid, trolox and gallic acid of water extract of fresh wheatgrass (Table 4) was 446.71, 345.92, 117.03 mg/100g, respectively. The water extract showed significantly higher (pd<0.01) TAC compared to the methanolic extract. TAC of raw cabbage reported by Miller et al. [22] was 150  $\mu$ mol Trolox equivalent/100g. TAC of raw spinach was reported to be 70.90 % [18].

**DPPH assay:** The mean percent inhibition of radical scavenging antioxidant assay of water and methanol extracts of wheatgrass is presented in table 5. The water extract showed significantly higher (pd<0.01) percent inhibition as compared to methanolic extract.

## DISCUSSION

The believers of alternative system of medicine claim that as chlorophyll and hemoglobin are alike in atomic structure; intake of wheat grass juice enhances hemoglobin production [23]. Kavita and Radhika [24] studied the effect of fresh wheatgrass juice supplementation on hemoglobin levels of anemic young women. The hemoglobin increased by 11.66 % after wheatgrass juice feeding for one month. In the present study, feeding fresh wheat grass juice for one month showed a higher increase in hemoglobin concentration compared to what was reported by kavita and Radhika [24]. Velmurugan et al. [25] reported a significant increase in liver and stomach glutathione in swiss albino mice upon feeding tomatoes at a level of 0.5, 1 and 2 g /kg of body weight.

Ingrid et al. [26] studied the plasma ascorbic acid concentration in response to spinach and broccoli soup feeding. The percentage increase was found to be 59 %. In the present study, the percentage increase in plasma ascorbic acid concentration was found to be 67.88 %. Thus after feeding fresh wheatgrass juice the percentage increase in blood ascorbic acid was higher than feeding spinach and broccoli soup as reported by the above mentioned authors.

Phenolic compounds tend to accumulate in the dermal tissue of plant body because of their potential role in protection against UV rays and a defense chemical against pathogen predators [27]. Total phenolic content contributes to antioxidant activity. The antioxidant capacity of phenol is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet O<sub>2</sub> quenchers, and metal chelators [28].

Flavonoids are a major class of total phenols. They are conjugated to sugar molecules and are commonly located in the upper epidermal layer of leaves. They are potent antioxidants and therefore they have been studied in the present investigation. The major phytochemical responsible for the antioxidants capacity most likely can be accounted for the flavonoids compounds, which are known as secondary natural product metabolites [29].

Thus, the results obtained in the present study support the fact that fresh wheatgrass juice helps in improvement of plasma antioxidant levels among geriatric population.

### ACKNOWLEDGEMENTS

The present research work was financially supported by UGC, New Delhi, India.

### REFERENCES

- [1] Wigmore, A: The wheatgrass book. Avery Publishing Group (1986).
- [2] Walters, R: The alternative cancer therapy book. Avery Publishing Group, New York, U.S.A. (1992).
- [3] Lai, C.N.: Nutr. Canc., 1: 19-21 (1979).
- [4] Peryt, B., Szymczyk, T. and Lesca, P.: Mutat. Res., 269: 201-215 (1992).
- [5] Gutteridge, J.M.C. and Halliwell, B.: Ann. N.Y. Acad. Sci., 899: 136-147 (2000).
- [6] Chatterjee, M.M. and Shnide, R.: Textbook of medical Biochemistry (fifth ed.), Jayvee Brother Medical Publishers (p) Ltd., New Delhi (2002).
- [7] Evently, K.A. and Mallay, H.T.: Jour. Bio. Chem., 126: 655-656 (1938).
- [8] Ellmen, G.L.: Arch. biochem, Biophys., 32: 8-12 (1959).
- [9] Roe, J. H. and Kuether, C. A.: J. Biol. Chem., 147: 399-400 (1943).
- [10] Benzie, I.F.F. and Strain, J.J.: Anal. Biochem., 239: 70-76 (1996).
- [11] Neeld, J.B. and Pearson, W.N.: J. Nutr., 79: 454-462 (1963).
- [12] Desai, J.D.: Vitamin E analysis in animal tissue. In: *Methods in Enzymology* (Academic Press), Orlando, FL, U.S.A (1984).
- [13] Rangana, S.C.: *Manual of Analysis of Fruit and Vegetable Products*. Tata McGraw Hill Publishing Company Limited, New Delhi (1979).
- [14] Schwarz, K., Bertelsen, G., Nissen, L., Gardner, P., Heinonem, M., Hopia, A., Huynh-Ba, T., Lambelet, P., McPhail, D., Skibsted, L. and Tijburg, L.: Euro Food Res. Technol., 212: 319-328 (2001).
- [15] Zhishen, J., Mengcheng, T. and Jianming, W.: Food Chem. 64: 555-559 (1999).
- [16] McCue, P. and Shetty, K.: Food Biotechnol., 17(1): 27-37 (2003).
- [17] Fletcher, R.H. and Fairfield, K.M.: J. Am. Med. Asso., 287: 3127-3129 (2002).
- [18] Gitanjali, Yasoda devi P. and Shiva prakash, M.: J. Food Sci. Tech., 6 (41): 666-668 (2004).
- [19] Vinson, J.A., Hao, Y., Su, X. and Zubik, L.: J. Agri. Food Chem., 46: 3630-3634 (1998).
- [20] Karadeniz, F., Burdurly, H.S., Koca, N. and Soyer, Y.: Turk J. Agric., 29: 297-303 (2004).
- [21] Chun, O. K., Smith, N., Sakagawa, A. and Lee, C.Y.: Int. J. Food Sci. Nutr., 3(55): 191-199 (2004).
- [22] Miller, H.E., Rigelof, F., Maraquart, L., Prakash, A. and Kanter, M.: J. Ame. Coll. Nutr., 19: 312S- 319S (2000).
- [23] Ben-Arye, E., Goldin, E., Wengrower, D., Stamper, A., Kohn, R. and Berry, E.: Scand. J. Gastroenterol., 37 (4): 444-449 (2002).
- [24] Sharma, K. and Shrivastav, R.: Applied Nutr., 26: 30-38 (2001).
- [25] Velmurugan, B.: Nutr., 28: 812-816 (2004).
- [26] Kiefer, I., Prock, P. and Lawrence, C.: J. Am. Coll. Nutr., 23(3): 205-211 (2004).
- [27] Toor, R.K. and Savage, G.P.: Food Res. Intern., 38: 487-494 (2005).
- [28] Rice Evans C.A., Miller, N.J. and Pagana, G.: (1995). Trends Plant Sci., 4: 304-309 (1995).
- [29] Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G. and Mainland, C.M.: J. Agri. Food Chem., 46: 2686-2693 (1998).