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Research Article

Effect of different cooking methods on the antioxidant properties of methanolic extract of beetroot (*Beta vulgaris* L.)

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ABSTRACT

Beet root was cooked using three different home scale cooking methods (boiling, pressure cooking and stir frying). The antioxidant content of flavonoids, tannins and polyphenols were estimated. The antioxidant activity was determined by ferric reducing antioxidant power assay and phosphomolybdenum assay. Results indicated that the total flavonoid, tannins and total polyphenols of raw beet root were 0.31g CE/ 100g (DWB), 0.25 g TAE/ 100 g (DWB) and 0.76 g GAE /100g (DWB) respectively. Cooking methods significantly increased the antioxidant content and antioxidant activities. Boiling exhibited the highest value among the three cooking methods. The antioxidant contents significantly correlated with antioxidant activities and also the correlation between FRAP and phosphomolybdenum assay was high.

Keywords: Antioxidants; Beetroot; Total Flavonoids; Tannins; Total Polyphenols; FRAP; Phosphomolybdenum assay

INTRODUCTION

Vegetables, as a group, contribute fibre, minerals and vitamins to the diet. Apart from their nutritive value, vegetables probably do more than any other group of foods to add appetizing colour, texture and flavour to our daily food. The Indian population is mostly vegetarian. Vegetables form an essential item of food both for the rich and the poor (Manay and Shadaksharaswamy, 2008). Fruits and vegetables contain many hundreds of compounds with potential antioxidant activity, including the antioxidant vitamin C and E, carotenoids, chlorophylls and a wide variety of antioxidant phytochemicals such as simple phenolic compounds, flavonoid glycosides and in some foods, complex polymeric tannins (Pellegrini *et al.* 2007). Flavonols and flavones are flavonoids of particular importance because they have been found to possess antioxidant and free radical scavenging activity in foods (Amic, *et al.*, 2003). Many phenols, such as flavonoids, have antioxidant capacities that are much stronger than those of vitamin C and E. *Beta vulgaris* L. (beet root) is ranked among the ten most powerful vegetables with respect to antioxidant capacity (Kähkönen, 1999; Vinson, 1998). It contains a significant amount of phenolics: catechin hydrate, epicatechin, ferulic, procatechuic, vanillic, *p*-

coumaric, *p*-hydroxybenzoic, caffeic and syringic acids (Kujala, 2000; Georgiev, 2010). Beetroot is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which are composed of two main groups, the red betacyanins and the yellow betaxanthins. In addition to their red color, betalains possess several desirable biological activities, including antioxidant, anti-inflammatory, hepatoprotective, and anti-tumor properties (Escribano, 1998; Kapadia, 2003; Winkler 2005). They are free radical scavengers and prevent oxygen – induced and free radical mediated oxidation of biological molecules (Pedreno and Escribano, 2001). Since many degenerative human diseases have been recognized as being a consequence of free radical damage, there have been many studies undertaken on how to delay or prevent the onset of these diseases. The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits. Epidemiological evidence has clearly shown that diets based on fruits and vegetables, with high content of natural antioxidants, contribute to reduced mortality from cardiovascular and cerebrovascular disease (Alia *et al.*, 2003). Vegetables usually undergo some type of processing before being ingested. The literature has shown that domestic cooking can result in significant losses in the composition and bioavailability of antioxidant compounds, such as ascorbic acid and some carotenoids are very sensitive to heat and storage. In contrast, polyphenols and flavonoids have shown certain stability when exposed to high temperatures, a quality that is reflected in the preservation of their antioxidant capac-

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ity (Azizah *et al.*, 2009). To obtain high quality food beneficial for maintaining good health, the effect of processing and cooking of vegetables on their radical scavenging activity must be understood. Hence the present study was undertaken to study the effect of cooking on the antioxidant contents and activity of beet root.

MATERIALS & METHODS

Beet root was purchased from the local market. About 2 kg of it was collected and washed in tap water followed by distilled water. These were peeled and inedible parts removed manually with a sharp knife, weighed and sliced to a uniform size of approximately 1cm cubes and well mixed and divided into four portions (100 g for each application). One portion retained raw, others were cooked in three different methods in triplicate, as given below. The beet root was cooked till it was just soft.

Boiling

Beetroot (100 g) was added to the 120 ml boiling water (distilled water) in a stainless steel vessel which was then covered with a lid to prevent water loss and cooked on a moderate flame at 100 °C for 10 min. It was drained off and cooled to room temperature for 10 min.

Pressure cooking

Beetroot (100 g) were placed in a stainless steel vessel containing 30 ml of distilled water and kept inside the cooker and pressure cooked for 5 min. It was drained and cooled to room temperature for 10 min.

Stir frying

Beetroot (100 g) was placed in a frying pan with 5 ml of hot refined groundnut oil, stir fried for 2 min and 70 ml of distilled water was added and covered with a lid to prevent water loss and cooked on a low flame for 8 min. The vegetable was cooled to room temperature for 10 min.

Preparation of extract

One gram of raw or cooked beetroot in 30 ml of methanol was homogenized in a blender and homogenate recovered. Methanol (15 ml) was used to wash the blender, pooled with the first homogenate and the mixture was centrifuged at 4500 rpm for 15 min at room temperature. Supernatants were used for the analysis of antioxidant components and antioxidant activity.

Chemicals and reagents

All the chemicals used were of analytical grade and deionised water was used for entire analysis. Sodium nitrite, aluminium chloride, Folin – ciocalteu reagent, sodium carbonate, gallic acid, tannic acid, 2,4,6-tripyridyl-S-triazine (TPTZ), butylated hydroxyl toluene,

ascorbic acid, catechin and sodium nitrite were purchased from SD Fine Chemicals Limited, India.

Determination of total flavonoids

Total flavonoids were measured using aluminium chloride colorimetric assay, as described by Marinova *et al.* (2005). Briefly, 1 ml of methanolic extract or standard solution of catechin (0.2 – 2.0 mg/ml) was added to test tubes containing 4 ml of double – distilled water. To the mixture was added 0.3ml 5 % NaNO₂. After 5 min, 0.3 ml 10 % AlCl₃ was added. After 6 min, 2ml 1 M NaOH was added and the total volume was made up to 10 ml with double – distilled water. The solution was mixed thoroughly and the absorbance of the samples, blank and standard were read at 510 nm using UV – Vis Double beam spectrophotometer 2201 Model. Total flavonoids content was expressed as g of catechin equivalent (CE) per 100 g on dry weight basis.

Determination of total polyphenols

Total phenolic contents were determined according to the spectrophotometric method of Sadasivam and Manickam (2008). Aliquots of (0.2 - 2 ml) of extracts were taken into test tubes and made up the volume to 3 ml with distilled water. To this 0.5 ml of Folin – ciocalteu reagent was added and mixed well. After 3 min, 2 ml of 20 % sodium carbonate was added and mixed well again. The test tubes were placed in a boiling water bath for exactly one min, cooled and measured the absorbance at 650 nm against a reagent blank. The total polyphenol content was calculated from the standard calibration curve obtained from gallic acid (10 - 120 µg/ml) and expressed as g of gallic acid equivalent (GAE) per 100 g on dry weight basis.

Determination of tannins

Tannin content was measured using Folin – Denis method (Sadasivam and Manickam 2008). One ml of appropriately diluted methanolic extract was transferred to a 100 ml volumetric flask containing 75 ml water. Five ml of Folin – Denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml with distilled water. The absorption of these solutions was measured at 700 nm after 30 min. The tannin content was calculated from the standard calibration curve obtained from tannic acid (10 – 100 µg/ml) and expressed as g of tannic acid equivalent (TAE) per 100 g on dry weight basis.

Ferric Reducing Antioxidant Power assay (FRAP)

Ferric reducing power (FRAP) was determined in the sample extracts according to Benzie and Strain (1999). The ability of the extracts to reduce ferric ions was determined in this assay. An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe(III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe(II)-TPTZ) complex. Briefly, 3.0 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ

Table 1: Antioxidant contents in fresh and cooked beetroot

Cooking methods	Total flavonoids CE g/ 100g (DWB)	Tannins TAE g/ 100g (DWB)	Total Polyphenols GAE g/ 100g (DWB)
Raw	0.31 ± 0.02 ^a	0.25 ± 0.0 ^a	0.76 ± 0.02 ^a
Boiled	2.17 ± 0.06 ^b	1.28 ± 0.03 ^b	3.56 ± 0.05 ^b
Pressure cooked	1.39 ± 0.03 ^c	0.76 ± 0.04 ^c	2.27 ± 0.02 ^c
Stir fried	1.15 ± 0.02 ^d	0.69 ± 0.02 ^d	1.95 ± 0.03 ^d

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$). DWB- Dry weight basis.

Table 2: Antioxidant activity in raw and cooked beetroot using FRAP and Phosphomolybdenum assay

Cooking methods	FRAP		Phosphomolybdenum assay	
	AAEAA g/ 100g (DWB)	BHAEAA g/ 100g (DWB)	AAEAA g/ 100g (DWB)	BHAEAA g/ 100g (DWB)
Raw	0.36 ± 0.03 ^a	0.36 ± 0.01 ^a	2.36 ± 0.04 ^a	3.54 ± 0.02 ^a
Boiled	0.83 ± 0.01 ^b	0.79 ± 0.04 ^b	5.61 ± 0.52 ^b	8.84 ± 0.04 ^b
Pressure cooked	0.53 ± 0.02 ^c	0.50 ± 0.02 ^c	3.65 ± 0.03 ^c	5.48 ± 0.04 ^c
Stir fried	0.47 ± 0.01 ^d	0.44 ± 0.01 ^d	2.42 ± 0.02 ^d	3.64 ± 0.01 ^d

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($p \leq 0.05$). DWB- Dry weight basis.

solution and 1 part of 20 mM Fe Cl₃.6H₂O solution) was added to the appropriate concentration of methanolic extract of beet root and the reaction mixture was incubated in a water bath at 37°C. The increase in absorbance at 593 nm was measured after 30 min. Ascorbic acid (1 - 10 µg/ ml) and butylated hydroxy anisole (BHA) (1 - 10 µg/ ml) were used as standard, and the results were reported as g of ascorbic acid equivalent of antioxidant activity (AAEAA) per 100 g (DWB) and g of BHA equivalent of antioxidant activity (BHAEAA) per 100 g (DWB).

Phosphomolybdenum assay

The total antioxidant capacities of the beet root extracts were evaluated by the phosphomolybdenum method as described by Prieto, Pineda and Aguilar (1999). In the presence of the extracts, Mo (VI) is reduced to Mo (V) and forms a green-coloured phosphomolybdenum V complex. The reagent solution was prepared by mixing 10 ml of 0.6 M sulphuric acid, 10 ml of 28 mM sodium phosphate and 10 ml of 4 mM ammonium molybdate into a beaker and 3 ml of reagent solution was added to all the test tubes. Methanolic extract of beet root was diluted with methanol to an appropriate concentration which was then added to 3 ml of reagent solution in test tubes. A typical blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample. All the tubes were capped and incubated in a boiling water bath at 95°C for 90 min. The tubes were cooled to room temperature and the optical density was measured at 695 nm against blank using UV - Vis spectrophotometer. Ascorbic acid (30 - 150 µg/ ml) and BHA (20 - 200 µg/ ml) was used as standard, and the results were reported as g of AAEAA per 100 g (DWB) and g of BHAEAA per 100 g (DWB).

Statistical Analysis

Experimental results were expressed as mean ± standard deviation. All measurements were replicated three times. Statistical analysis was done using SPSS Version 16. One way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out. Significance was accepted at $p \leq 0.05$. The correlation coefficient (r) for the relation between the antioxidant activity and the antioxidant contents was determined using Pearson correlation coefficient.

RESULTS AND DISCUSSION

The effect of cooking methods on the antioxidant content of beet root

The total flavonoids, tannins and total polyphenol contents of the raw and cooked beet root are given in Table 1. The total flavonoid content ranged from 0.31-2.17 g CE/ 100g DWB, tannins 0.25 - 1.28 g TAE/ 100 g and total polyphenols 0.76 - 3.56 GAE /100g. On cooking by the three methods, the total flavonoids, tannins and total polyphenols significantly ($p \leq 0.05$) increased to various extent, depending on the type of cooking method. The increase was in the following order: boiling > pressure cooking > stir frying > raw. The results are consistent with Bellail *et al.* (2012) who indicated an increase in the phenolic contents of sweet potato cultivars after cooking methods such as boiling, baking, microwave cooking and deep frying. They reported that heat processing caused cell structure damage which resulted in increasing the efficiency of extraction. Turkmen *et al.* (2005) also reported that the total phenolic contents were increased in pepper (114 %), green beans (114 %), broccoli (94 %) and spinach (101 %) after boiling for 5 min. The results of the present study are in agreement with that reported by Adefegha

and Oboh (2011). They reported that most of the phenolic compounds trapped in fibre of green leafy vegetables are actually more available on cooking than the raw and the total flavonoids of cooked vegetables were higher than raw, indicating a possible release of flavonoids during the cooking. According to Oboh (2005) blanching of green leafy vegetables in boiled water for 5 min caused a significant increase ($p \leq 0.05$) in polyphenols. These results suggest that some common cooking treatments can be used to enhance the nutritional value of vegetables and increasing bioaccessibility of health promoting compounds.

The effect of cooking methods on the total antioxidant activity of beet root

In the present study the total antioxidant activity of beet root significantly ($p \leq 0.05$) increased on cooking compared to the values for the raw ones (Table 2). Antioxidant activity of beet root as determined by the FRAP method and phosphomolybdenum assay decreased in the order: boiling > pressure cooking > stir frying > raw. Turkmen *et al.* (2005) also stated that there was a significant increase in the antioxidant activity in pepper, green beans, broccoli and spinach after boiling or 5 min, which is in agreement with the present report. Bellail *et al.* (2012) reported that all cooking methods showed significant increase in reducing power values and DPPH values. The antioxidant activity demonstrated by the cooked pumpkin was found to decrease in the following order of boiled 2 minutes > boiled 4 minutes > boiled 6 minutes > stir-fried 2 minutes > stir-fried 4 minutes > stir-fried 6 minutes > fresh pumpkin (Azizah *et al.* 2009). According to Miglio *et al.* (2008) fried vegetables showed the lowest degree of softening, even though antioxidant compounds were less retained. An overall increase of trolox equivalent antioxidant capacity, total radical – trapping antioxidant parameter and ferric reducing antioxidant power values was observed in all cooked vegetables, probably because of matrix softening and increased extractability of compounds. Ferracane *et al.* (2008) found that the antioxidant capacity of cooked artichokes, measured by three different assays, enormously increased after cooking, particularly after steaming (up to 15- fold) and boiling up to (8 – fold). The cooking effect observed on the artichoke antioxidant profile is probably due to matrix softening and increased extractability of compounds.

Relationship between antioxidant contents and antioxidant activity

The total flavonoids, tannins and total polyphenols correlated significantly with antioxidant activities. As shown in Figures 1 to 6 there was a strong significant correlation between AAEEA and total flavonoids ($r = 0.949$; $r^2 = 0.900$), AAEEA and tannins ($r = 0.964$; $r^2 = 0.930$), AAEEA and total polyphenols ($r = 0.960$; $r^2 = 0.922$), BHAEEA and total flavonoids ($r = 0.929$; $r^2 = 0.862$), BHAEEA and tannins ($r = 0.945$; $r^2 = 0.893$),

BHAEEA and total polyphenols ($r = 0.944$; $r^2 = 0.890$) determined using FRAP method. Similarly as shown in Figures 7 to 12 a strong significant correlation was found between AAEEA and total flavonoids ($r = 0.896$; $r^2 = 0.802$), AAEEA and tannins ($r = 0.909$; $r^2 = 0.826$), AAEEA and total polyphenols ($r = 0.911$, $r^2 = 0.830$), BHAEEA and total flavonoids ($r = 0.896$, $r^2 = 0.802$), BHAEEA and tannins ($r = 0.909$, $r^2 = 0.827$), BHAEEA and total polyphenols ($r = 0.912$, $r^2 = 0.831$) determined using phosphomolybdenum assay. There was a high correlation between the antioxidant activities determined by FRAP method and phosphomolybdenum assay. The findings of the present study are consistent with the results reported by Olajire and Azeez (2011). They found that there was a good linear correlation ($r^2 = 0.861$, $p < 0.05$) between the total phenolic content and the scavenging of DPPH radical in vegetable extract. According to Bellail *et al.* (2012) total phenolic contents were significantly correlated with the reducing power values ($r = 0.988$, $p \leq 0.0001$) and DPPH activities ($r = 0.926$, $p \leq 0.0001$). Chun *et al.* (2004) observed that there was a good linear relationship between the total phenolics and antioxidant capacity ($r^2 = 0.9743$) and between the total flavonoids and antioxidant capacity ($r^2 = 0.9557$) in raw and processed cabbages.

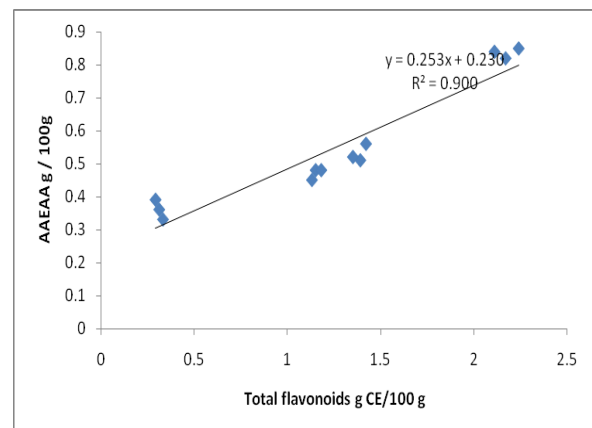


Figure 1: Relationship between AAEEA and total flavonoid contents of beetroot (FRAP)

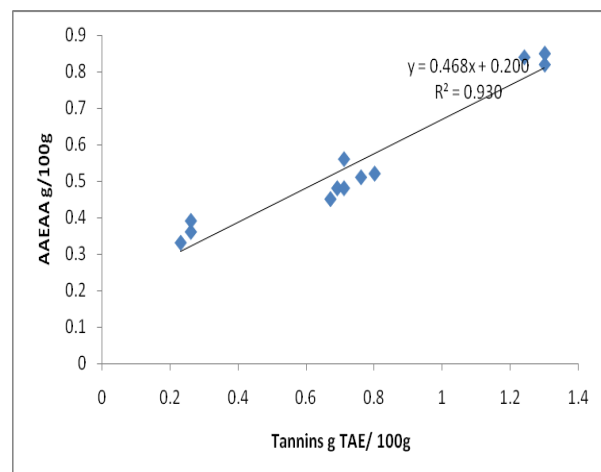


Figure 2: Relationship between AAEEA and tannin contents of beetroot (FRAP)

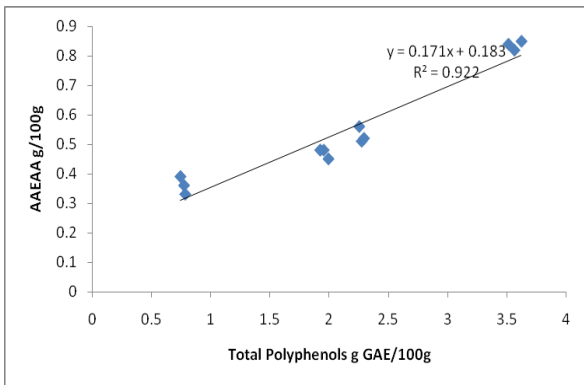


Figure 3: Relationship between AAEAA and total polyphenol contents of beetroot (FRAP)

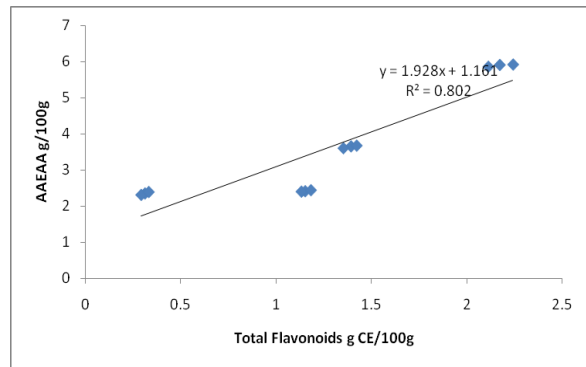


Figure 7: Relationship between AAEAA and total flavonoid contents of beetroot (Phosphomolybdenum assay)

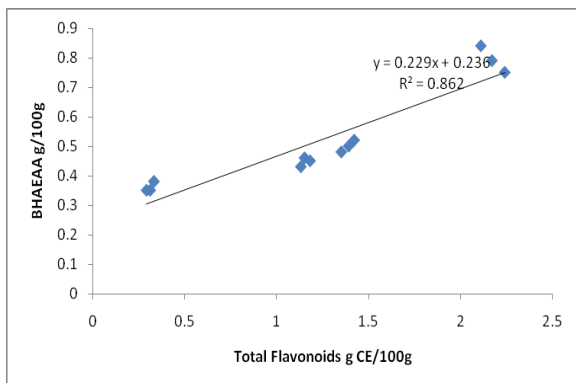


Figure 4: Relationship between BHAEAA and total flavonoid contents of beetroot (FRAP)

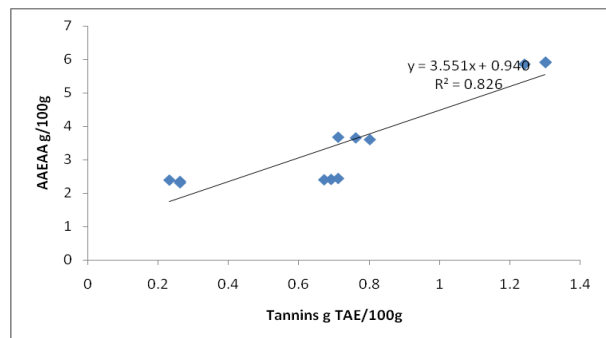


Figure 8: Relationship between AAEAA and tannin contents of beetroot (Phosphomolybdenum assay)

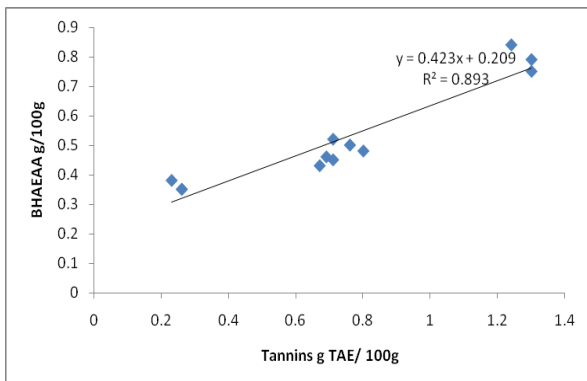


Figure 5: Relationship between BHAEAA and tannin contents of beetroot (FRAP)

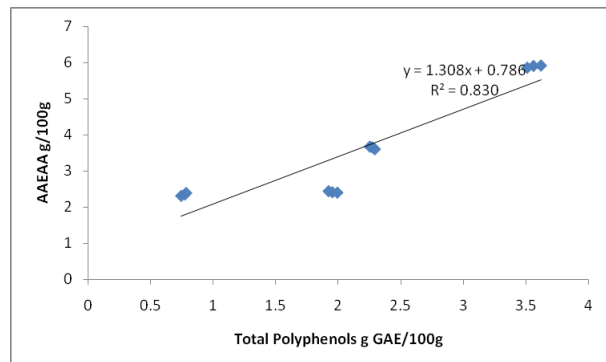


Figure 9: Relationship between AAEAA and total polyphenol contents of beet root (Phosphomolybdenum assay)

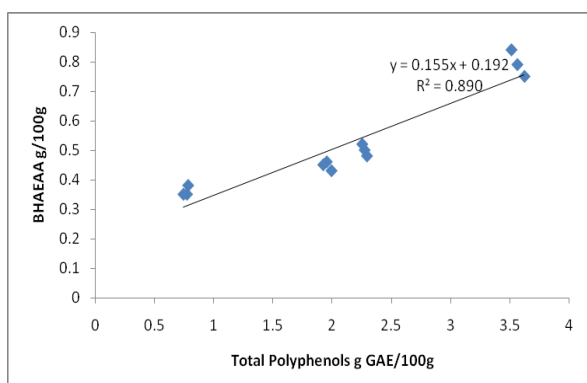


Figure 6: Relationship between BHAEAA and total polyphenol contents of beetroot (FRAP)

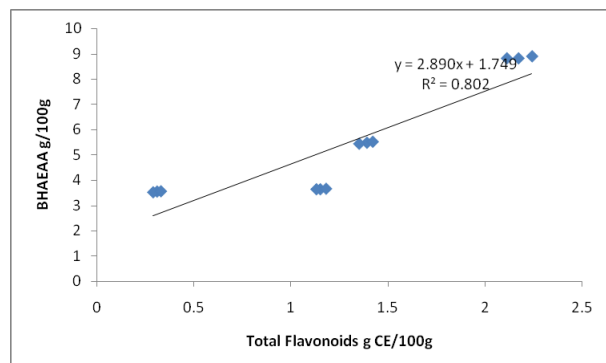


Figure 10: Relationship between BHAEAA and total flavonoid contents of beetroot (Phosphomolybdenum assay)

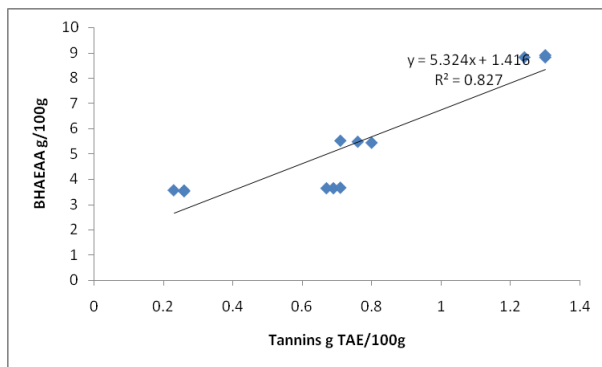


Figure 11: Relationship between BHAEEA and tannin contents of beetroot (Phosphomolybdenum assay)

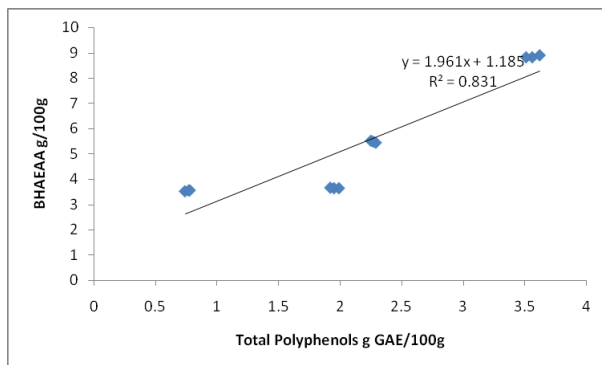


Figure 12: Relationship between BHAEEA and polyphenol contents of beetroot (Phosphomolybdenum assay)

CONCLUSION

This study confirms that beet root is a vegetable that has rich antioxidant properties and is valued for its health promoting features. It is inferred that simple cooking methods like boiling, pressure cooking and stir frying for minimum time helps to release the antioxidants with least degradation. Hence simple moist heating is the most favourable method of cooking such health promoting vegetables.

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