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Effect of Processing on Antioxidant Properties of Ber (*Zizyphus mauritiana)* Fruit

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Abstract: ***Indian Ber contain good source of ascorbic acid and total phenolics. Despite being rich in antioxidants it is an underutilized fruit. Effect of processing on antioxidant activity of ber fruit was studied. Antioxidant activity of ber fruit was evaluated by different methods viz. DPPH radical activity, reducing power assay, superoxide anion radical activity, TBARS, total phenolic content and total flavonoid conent. The results indicated that fresh ber fruits has 78.57±0.16% inhibition DPPH scavenging radical activity, 3.51±0.05 absorbance reducing power activity, 74.0±2% super oxide anion radical activity, 232.84±3.06% TBARS activity, 94.7±0.27 (µg of PE) total phenolic content and 7.48±0.01 µg of RE total flavonoid content. Blanching of ber fruits enhanced the total flavonoid content and super oxide anion radical activity but, at the same time, it reduce the scavenging radical activity, reducing power activity and total phenolic content compared to fresh fruit. Hence, optimization of blanching time and temperature for ber fruits is necessary. Secondary processing of ber fruits slightly slowed down the scavenging radical activity, reducing power activity, total flavonoid content and total phenolic content but raised the super oxide anion radical activity in RTS Ber beverage. TBARS activity of fruit increased 29% on blanching and 52% in RTS ber beverage. Therefore, value addition of ber fruits can boost the economy and health benefits by reducing the post harvest loss, establishment of agro process industry and promoting the importance of functional products from ber fruit.***

Keywords: Ber fruit, Blanching, RTS ber beverage, DPPH radical activity

1. Introduction

Ber is a tropical and subtropical fruit native to the northern hemisphere. It belongs to the genus *Ziziphus* of the family Rhamnaceae and order Rhamnales. There are two major domesticated jujubes, *Z. mauritiana* Lam. (Indian jujube or ber) and *Z. jujuba* Mill (Chinese or common jujube). Among two, former is commonly cultivated throughout the northwest of India and in the arid parts of South India (Azam *et al.,* 2001; Sunil *et al.,* 2009). It is popularly called the king of arid zone fruits (Yamadagni, 1985; Shoba and Bharathi, 2007). The area under cultivation with this fruit is 8.7lakh ha with an annual production of 8.9lakh tones in India (Baloda *et al.,* 2012). About 125 varieties of ber are available in India. A few of these varieties are known for taste, size, amount of pulp and higher yields. The cultivars Umran, Kathapal and Gola are the most promising varieties of ber in North India (Azam-Ali, 2001).

Ber fruits are highly nutritious, rich in ascorbic acid and contain fairly good amount of vitamin A and B, minerals like calcium, phosphorus and iron (Yamadagni, 1985; Shoba and Bharathi, 2007). Caffeic acid, *p*-hydroxybenzoic acid, ferulic acid and *p*-coumaric acid are predominant phenolics reported in ber (Tanmay *et al.,* 2011; Ayaz *et al.,* 2012) which account for its significant antioxidant activity, reducing power activity and scavenging of free radical activity (Krishna and Parashar, 2012). Indian Ber contain good source of ascorbic acid and total phenolics ranging from 19.54 to 99.49 mg/100g and 172 to 328.6 mg GAE/100g respectively (Koley *et al.,* 2011) and average antioxidant activities were 1.6–6.33 and 1.22 –5.49 µ mol TE/g as the CUPRAC and FRAP assays, respectively (Krishna and Parashar, 2012). The highest levels of polyphenol, tannin, glutathione, and ascorbic acid contents were reported in pulp of Chinees jujube (Taraneh and Asna, 2012). Since, ber is a seasonal fruit, rich in antioxidants and to make it available throughout the year for its nutritive value, different preserved products needs to be prepared. Ber has been widely used in folk medicines for treatment of allergies, constipation, insomnia, depression, chronic bronchitis, fever and enlargement of liver (Tanmay *et al.,* 2011).

Food processing involves changes in structural integrity of the plant material and this produces both negative and positive effects on their antioxidant activity. The antioxidant activity is diminished owing to inactivation of antioxidant compounds caused by different chemical reactions enhanced by the effect of heat. The positive effects of food processing include in some cases, transformation of antioxidants into more active compounds, such as the deglycosylation of onion quercetin, as well as an increase in the antioxidant activity owing to inhibition of enzymes (Jana *et al.,* 2007).

However not many studies have focused on the effect of minimal (blanching) and secondary processing (RTS ber beverage) on antioxidant properties in semi arid fruits such as ber. If data is available on the effect of processing on natural antioxidants in the fruit, it can be utilized to promote processing, thereby increasing the utilization, shelf life and consumption. (Wei-min Zhang *et al.,* 2009; Kosanic *et al.,* 2011). Therefore, our aim of research work is to evaluate the effect of processing on antioxidant properties of Ber fruit.

2. Material and Methods

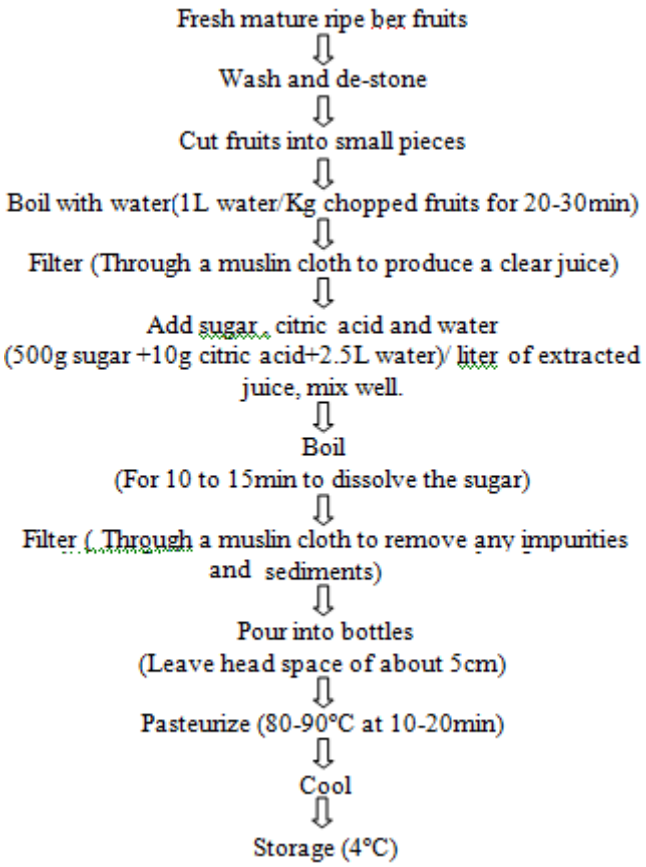
Procurement of Raw Materials

Mature ripe (yellowish-green color) ber fruits were procured from the local market and the cultivar was identified by the Taxonomy experts of the College of Horticulture, Rajendranagar, Hyderabad. They identified the cultivar as Gola. The procured fruits were washed in tap water and immersed in a detergent solution for two minutes, in order to remove the organic dirt from the fruit that comes from the field. The detergent Nitrol WV-2640 (brand Nippon) was

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used at a concentration of 1.0% (10 mL.L^{-1} water). After two minutes, the fruits were rinsed with tap water and immersed, for 15 minutes, in a chlorine solution at a concentration of 200 ppm in order to reduce the microbial load and then again washed thoroughly in clean water and blanched at 80^0C for 8 min in hot water. The detailed procedure of preparation of RTS Ber beverage is given in the flowchart. After blanching and secondary processing of ber fruits were subjected to antioxidant analysis.

3. Processing flow chart for the RTS Ber beverage (Azam-Ali, 2001; Helmy, 2012)



3.1 Preparation of Extract

Extract was prepared from 50g irradiated fruit and fresh fruit separately by using mixture of equal quantity of acetone methanol and water (250ml). The extract was centrifuged at 10,000rpm for 15min. The resulting supernatant was collected and the pellet re-extracted and the supernatants were pooled together. The filtered extract was used for analysis of Scavenging DPPH radicals, Reducing power, Superoxide anion radical scavenging activity, total phenolic compounds and total flavonoid content. Scavenging DPPH radicals, Reducing power, Superoxide anion radical scavenging activity, Total phenolic compounds and Total flavonoid content.

3.2 Scavenging DPPH radicals (Dorman *et al.*, 2004)

The free radical scavenging capacity of the extracts was determined using 1,1- diphenyl-2-picryl- hydrazil (DPPH). Two ml of methanol solution of DPPH radical in the concentration of 0.05 mg/ml and 1ml of extract were placed in cuvettes. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm against methanol as blank in spectrophotometer. The DPPH free radical concentration was calculated using the following equation:

DPPH scavenging effect (%) = (A_0 - A_1 / A_0) X 100

Where, A_0 was the absorbance of the negative control or blank and A_1 was the absorbance of reaction mixture or standards.

3.3 Reducing power (Oyaizu, 1986)

One ml of extract were mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were incubated at 50^0C for 20 min. Then trichloroacetic acid (10%, 2.5ml) was added to the mixture and centrifuged. Finally, the upper layer (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml; 0.1%). The absorbance of solution was measured at 700 nm in spectrophotometer. Blank was prepared with all the reaction mixture, indicated that the reducing power is increased.

3.4 Superoxide anion radical scavenging activity (Nishimiki *et al.*, 1972)

0.1ml of extracts was mixed with 1 ml nitro blue tetrazolium (NBT) solution (156 µM in 0.1 M phosphate buffer, pH 7.4) and 1ml NADH solution (468 µM in 0.1 M phosphate buffer, pH 7.4). The reaction was started by adding 100 µL of phenazine methosulphate (PMS) solution (60 µM in 0.1 M phosphate buffer, pH 7.4). The mixture was incubated at room temperature for 5 min and the absorbance was measured at 560 nm in spectrophotometer against blank samples.

The following formula was used to calculate the percentage inhibition of superoxide anion generation

Superoxide anion scavenging activity (%) = (A_0 - A_1/A_0) × 100

Where A_0 is the absorbance of the negative control consisting of all the reaction agents except the extract); A_1 is the absorbance of reaction mixture or standards.

3.5 Total phenolic compounds

Total soluble phenolic compounds in the ber fruit extract were determined with Folin-Ciocalteu reagent according to the method of Slinkard (Slinkard and Slingleton 1997) using pyrocatechol as a standard phenolic compound. Briefly, 1ml of the extract was diluted with 46 ml of distilled water. Then, one milliliter of Folin Ciocalteu reagent was added and the mixture was stirred vigorously. 3 ml of Na_2CO_3 (2%) was added after 3 min and then was allowed to stand for 2hr with intermittent shaking. After that, absorbance was measured at 760 nm in spectrophotometer against blank consisting of all the reaction agents except the extract. The total phenol content in the extract was determined as

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microgram of PE according to the equation that was obtained from standard pyrocatechol graph as

Absorbance = 0.0021×total phenols [µg pyrocatechol equivalent] - 0.0092

3.6 Total flavonoid content

The total flavonoid content was determined using the Dowd method (Meda *et al.,* 2005). Two ml of the extract solution was mixed with 2 ml of 2% aluminiumtrichloride ($AlCl_3$) in methanol. The mixture was incubated for 10 min at room temperature and the absorbance was measured at 415 nm in spectrophotometer against blank samples. The total concentration of flavonoids in the extracts was determined as microgram of REaccording to the formula that was obtained from standard rutin graph as

Absorbance = 0.0144×total flavonoid [µg rutin equivalent] + 0.0556

TBARS (Thiobarbituric acid reactive substances) method (Nikols *et al.* 1994)

This method selected for calculating the total antioxidant activity of ber fruit.

3.7 Sample preparation

One g sample was homogenized in a motor and pestle with 10 ml of 0.1M phosphate buffer (pH 7.8) and one percent of 0.05M EDTA and centrifuged at 4000 rpm for 15 minutes at 5ºC. The clear supernatant extract was used for analysis.

The reaction mixture contained 2.3g aliquot of sample, coconut oil (0.24 ml) in phosphate buffer (0.26) ml, 0.1M, pH 7.8), ferrous sulphate (0.05mM), ascorbic acid (0.4mM), potassium hydrogenpthalate 100mM, pH 6.0) BHT (25mM in 5 ml hexane) in a final volume of 2.4 ml. Content of the tube were incubated for 30 min at 37ºC. TCA (0.75 ml, 20%) was added and centrifuged at 10,000 rpm for 30 min at 4ºC, followed by addition of TBA (0.5% in 0.1N NaOH). Distilled water was added to equalize the final volume to 3.24 ml. This was heated at 95ºC in water bath for 30 minutes followed by immediate cooling in ice pack for 5 minutes. Finally the reaction mixture was submitted to read the absorbance at 532 nm against TBA activity.

4 Statistical Analysis

The results obtained were subjected to statistical analysis with the window STAT programme. To determine the statistical significance of antioxidant activity, Analysis of Variance (ANOVA) technique was used. Pearson's bivariate correlation test was used to calculate correlation coefficients between the content of total phenolic, total flavonoid content, Scavenging DPPH radical activity, Reducing power and superoxide anion radical scavenging

5 Results and Discussion

The results of scavenging DPPH radicals of studied ber fruits are summarized in the table.1 Higher % Inhibition indicates better scavenging activity or antioxidant potential. (Padmanabhan and Jangle, 2012). DPPH radical scavenging activity of ber fruits were studied in raw, blanched ber fruits and RTS Ber beverage. There was a significant ($p>0.05$) decrease in free radical activity on blanching (78.57±0.16 to 71.97±0.26% inhibition) as compared to raw fruit. It may be due to loss of vitamin C in the blanched fruits. Ganiyu (2005) was observed same in eight popularly known green leafy vegetables in Nigeria. The radical activity of RTS Ber beverage was significantly ($p>0.05$) decreased (69.76±0.20 % inhibition) as compared to raw fruit. Vithlani and Patel (2010) reported that the DPPH capacity was increased significantly in jujube wine (45%) and vinegar (76%) as compared to fresh jujube juice (22%).

Table 1: Effect of processing on antioxidant properties of ber

TREATMENTS	DPPH[1]	RP[2]	SOA[3]	TBARS[4]	TP[5]	TF[6]
Raw fruit	78.57^{a}±0.16	3.51^{a}±0.05	74.00^{c}±2.0	232.84^{c}±3.06	94.70^{a}±0.27	7.48^{b}±0.01
Blanched Fruit	71.97^{b}±0.26	1.28^{b}±0.06	88.22^{a}±0.38	300.49^{b}±3.06	81.21^{b}±0.27	15.17^{a}±0.00
RTS Ber beverage	69.76^{c}±0.20	1.21^{b}±0.01	85.11^{b}±0.0	354.44^{a}±1.87	18.50^{c}±0.04	2.78^{c}±0.02

Values are represented in Mean±SD at 5% significant level
[1]**DPPH** – 1, 1-diphenyl-2-picryl-hydrazil, activity is expressed in % inhibition. **S.Ed-** 0.22556,**CD**-0.62626***
[2]**RP** – Reducing power expressed in absorbance, SEd-0.04761, **CD**-0.13218**[4]
TBARS- SEd-2.29161, **CD**-6.36253***
[3]**SOA** – super oxide anion scavenging activity is expressed in %. **SEd**-0.83163,**CD**-2.30898***
[5]**TPand** [6]**TF** -total phenolics is expressed in µg pyrocatechol.**SEd**-0.22504, CD-0.62482*** and total flavonoid is expressed in µg rutin. **SEd** 0.02018,**CD**- 0.05604***

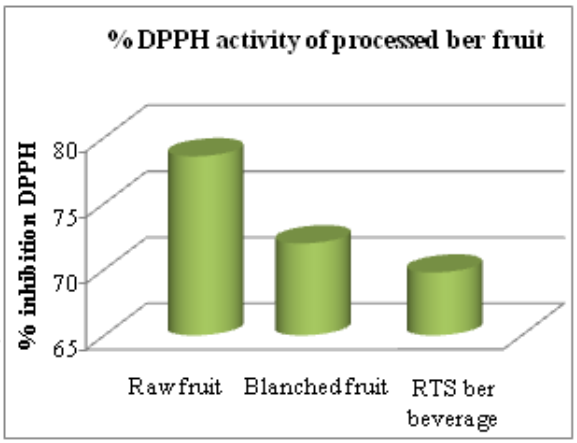


Figure 1: % Scavenging DPPH radical activity of processed ber fruits

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The reducing power activity was significantly ($p>0.05$) high in raw fruit as compared to blanching and RTS Ber beverage. The reducing power activity was significantly ($p>0.05$) declined from 3.51±0.05 absorbance to 1.28±0.06 absorbance during blanching as compared to raw fruit. Similar observation was found in RTS Ber beverage. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity. The reducing properties are generally associated with antioxidant action of reductones such as ascorbic acid. A study by Ganiyu (2005) reported that blanching for 5 min. cause a significant ($p\leq0.05$) decreased in vitamin C content, reducing power and free radical scavenging ability of eight popularly consumed green leafy vegetables in Nigeria.

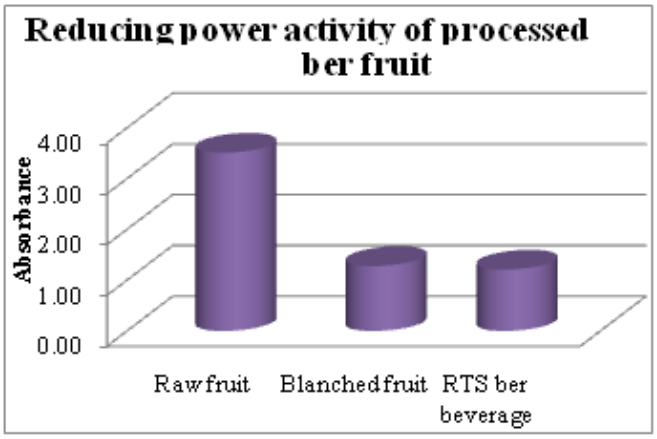


Figure 2: Reducing power activity of processed ber fruits

The super oxide anion radical scavenging activity has significantly ($p>0.05$) increased during blanching (88.22±0.38 %) and RTS Ber beverage (85.11±0 %) as compared to raw fruit (74.0± 2 %). Superoxide's are produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as auto-oxidation by catecholamine (Sagar *et al.,* 2011).

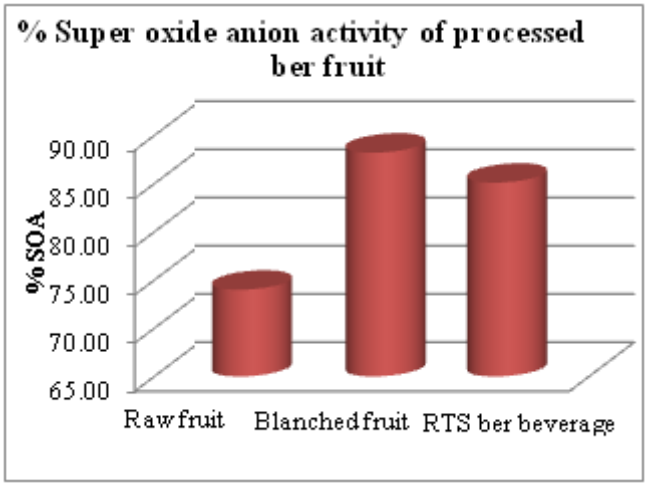


Figure 3: % Super oxide anion radical activity of processed ber fruits.

The per cent TBARS and MDA are inversely proportional to antioxidant activity *i.e.*, with increase of TBARS content, there will be decrease in antioxidant activity of fruit. TBARS activity of raw ber fruit was found to be 232.84±3.06% which has significantly ($p>0.05$) increased in blanched fruits and RTS Ber beverage 300.49±3.06% and 354.44±1.87%) as compared to raw fruit. It indicates that the total antioxidant activity of ber fruits decreased on processing.

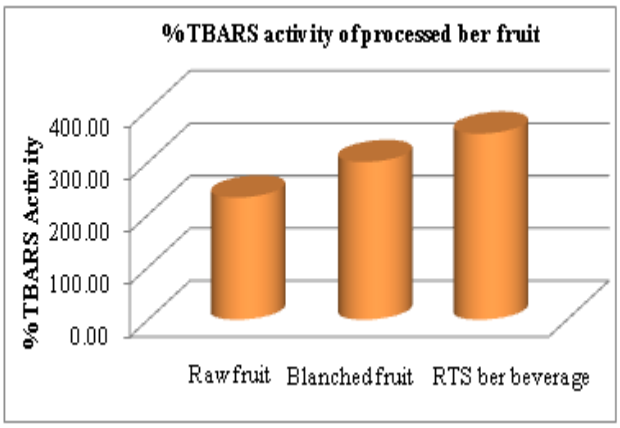


Figure 4: % TBARS activity of processed ber fruits

The antioxidant activity of phenolic compounds is mainly attributed to their redox actions, neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The results of total phenolic content of investigated ber fruits are presented in fig 5. The total phenolic content of fresh fruit was found to be 94.7±0.27 (µg of PE) which has significantly ($p>0.05$) declined to 81.21 ± 0.27 (µg of PE) on primary processing *i.e.,* blanching. The phenolic content in the jujube was different with cultivars and different parts of fruits such as peel and pulp (Zipin *et al*., 2009). Caffeic acid and *p*-coumaric acid are the major phenolic compounds in ber (Ayaz *et al.,* 2012) these compounds were reduced at temperatures higher than 60°C in apple (Maria *et al.,* 2012). In most studies, results of heat treatment on the total phenolic content are contradicting. Some researchers reported increase in the phenolic content whilst others observed a decrease (Chipurura *et al.,* 2010). Total phenolic content of selected vegetables (peas, carrot, spinach, cabbage, cauliflower, yellow turnip and white turnip) was found to be generally decreased by boiling, frying and microwave cooking (Sultan *et al.,* 2008). A study by Amin *et al.,* (2006) reported blanching up to 15 min was found to cause losses of phenolic content in spinach, it may be vary depending on the species of spinach.

The total phenolic content in RTS Ber beverage has significantly ($p>0.05$) decreased 18.50±0.04 (µg of PE) as compared to raw fruit. The RTS Ber beverage was prepared with ber pulp which was filtered through muslin cloth. If the pulp was not filtered, then the phenolic content would have been more as per the studies by Candrawinata *et al.* (2012). The extraction technique adopted for juice production can make significant difference to the quality of the juice in terms of its antioxidant composition. Enzyme-assisted processing can significantly improve the functional properties of the *Zizyphus* juice (Tanmay *et al.,* 2011)

Total flavonoid content of blanched ber fruits had shown significant ($p>0.05$) increase (15.17 ± 0 µg of RE) as compared to raw fruit. Similarly, blanching increased the

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flavonoid content in mango fruits (Nohime *et al.,* 2012). It may be due to phytochemicals, which contain non polar secondary metabolites, remain almost inactive but the heat treatment may affect antioxidant properties due to release of phenolic phytochemicals (Oboh *et al.,* 2010). Total flavonoid content (µg of RE) of RTS Ber beverage has shown significant decrease (2.78 ± 0.02) as compared to raw fruit with statistical significance. Addie *et al.* (2002) reported that the levels of flavonoids and chlorogenic acid in the juice were reduced to between 50% (chlorogenic acid) and 3% (catechins) and most of the antioxidants were retained in the pomace. Hence, the RTS Ber beverage has lower total flavonoid content than fresh fruit.

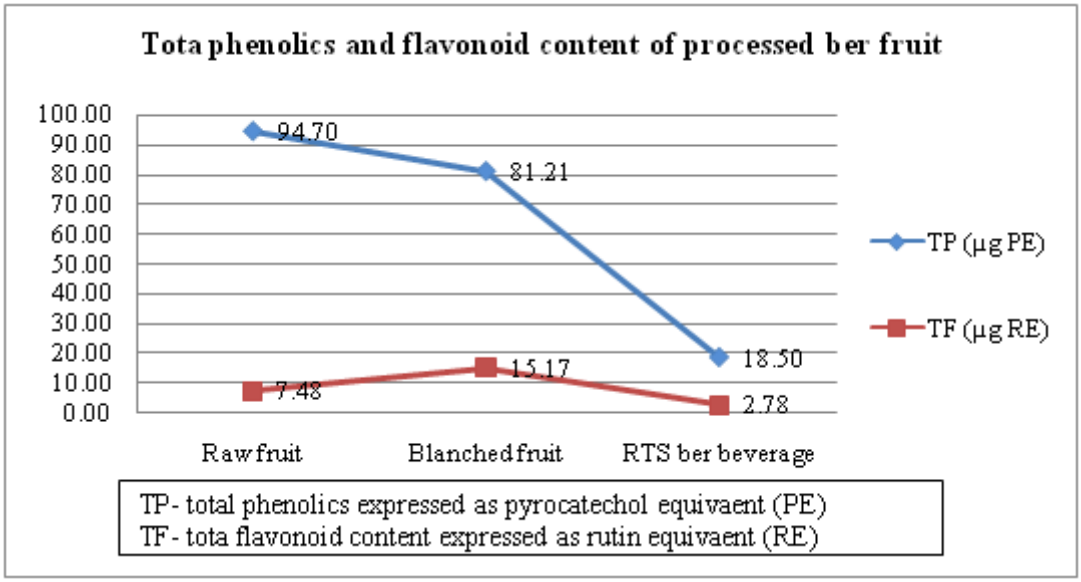


Figure 5: Total phenolics and flavonoid content of processed ber fruits.

Table 2: Pearson's correlation coefficients of antioxidant activities of ber fruit on processing

	[1]DPPH	[2]RP	[3]SOD	[4]TP	[5]TF
[1]DPPH	1				
[2]RP	0.98**	1			
[3]SOD	-0.90*	-0.97**	1		
[4]TP	0.80*	0.66	-0.46	1	
[5]TF	0.10	-0.11	0.34	0.68	1

[1]DPPH - 1, 1-diphenyl-2-picryl-hydrazil, activity is expressed in % inhibition. **[2]RP** - Reducing power expressed in absorbnce, **[3]SOA** - super oxide anion scavenging activity is expres sed in %. **[4]TP**-total phenolics is expressed in µg pyrocatechol**. [5]TF**- Total flavonoid is expressed in µg rutin.**ns-** non significant **,** * **-** Significant (p>0.05) , ** **-** Significant (p>0.01)

Total phenolic content was strongly correlated (r=0.80) with DPPH radical activity in processed ber fruits.

6 Conclusion

Study reckoned that blanching of ber fruits enhanced the total flavonoid content but, simultaneously it reduced the scavenging radical activity, reducing power activity, total phenolic content of fruit. Hence, optimization of blanching time and temperature for ber fruits is necessary. Secondary processing of ber fruits slightly reduced the scavenging radical activity, reducing power activity, total flavonoid content, total phenolic content but it raised the super oxide anion radical activity and TBARS activity in RTS Ber beverage. Antioxidant properties retained during processing of ber fruits helps to establishment of agro process industry and promoting the importance of functional products from ber fruit. It results boost the Indian economy as well as health benefits.

7 Future Scope

Some *in vivo* studies have been done on antioxidant activity of ber fruits but not on ber products. Therefore further research can be done on *in vivo* studies of antioxidant activity in ber products. Study of antioxidant properties in cv. Umran, Gola, Illaichi ber fruit has been done but there are some wild varieties available in different parts of India which require the light of research on their antioxidant properties. Hence similar study on available wild varieties could lead to more untapped sources of functional ingredients.

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