aerobic conditions in order to maintain its quality. Long term storage studies should be conducted to investigate the optimum timing and storage conditions of vermicompost for enhanced benefits in field application.

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A comparative study of antioxidant activity and total phenolic content of fresh juices of some common Indian fruits with their commercial counterparts

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Oxidative stress caused by overproduction of free radicals has been implicated in the pathogenesis of various chronic diseases like cancer, diabetes, cardiovascular diseases, and neurodegenerative and immunological disorders. Physiologically, these free radicals are scavenged continuously by numerous beneficial substances known as antioxidants. Fruits are rich in antioxidants such as ascorbic acid, flavonoids and polyphenols that strengthen our immunity and help us maintain good health. In recent times, there has been an increasing trend to supplement our diet with packaged fruit juices. In light of this, the present study aims to compare the antioxidant activity and total phenolic content (TPC) of commonly available fresh juices of some fruits found in the Indian subcontinent with their commercial counterparts, available in two popular brands. Folin–Ciocalteu method was used to determine TPC, while ferric reducing antioxidant power assay was performed to evaluate the antioxidant activity of fruit juices. Among fresh juices, the highest antioxidant property and TPC was found in pomegranate followed by litchi. Amongst packed fruit juices, the antioxidant property and TPC was highest in pomegranate and lowest in apple. However, when compared with fresh fruit juices, the antioxidant activity as well as TPC of commercial juices were observed to be significantly less in all cases. These observations prompt serious rethinking on the use of commercial juices as a source of antioxidants.

Keywords: Antioxidant activity, fresh fruits, packaged juices, total phenolic content.

REACTIVE oxygen species are known to cause various types of damages to biological systems leading to many non-communicable diseases[1–3]. Several epidemiological studies as well meta-analysis have revealed the protective effect of fruit juices against cancer, stroke and other non-communicable diseases that can be related to the antioxidants found in these fruits. These antioxidants play a crucial role in the maintenance of health and prevention of various pathological conditions such as cardiovascular and neurological diseases, age-related disorders, cancer, etc.[4–6]. A report published in 2003 by the World Health

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Organization (WHO) on diet, nutrition and prevention of chronic diseases mentions that insufficient intake of fruits and vegetables is estimated to cause around 14% of all gastrointestinal cancer deaths, about 11% of ischaemic heart disease-related deaths and about 9% of stroke-related deaths. According to this report, low fruit and vegetable intake is also among the top 10 risk factors contributing to overall mortality[7]. Furthermore, WHO recommends a daily intake of at least 400 g of fruits (or five daily servings with an average serving size of 80 g). Surprisingly, even though India is among one of the largest producers and exporters of fruits, a study based on secondary information analysis and survey of 1001 consumers spread across many states reports that only 21.2% of Indians consume fruits and vegetables[8].

Moreover, in developing countries like India, people are witnessing a sudden change in their lifestyles and thus adopting new instant food habits. With rapid urbanization, surge in income and less availability of time, more and more consumers are relying on packaged fruit juices in comparison to fresh fruits. This trend has driven the food industry to develop new functional foods and beverages with added health benefits. Antioxidants in foods and beverages have become increasingly popular because of their potential health benefits as advertised by their manufacturers. According to a recent report, the branded fruit juice market in India is estimated to be around Rs 1100 crores. However, there are several disadvantages of choosing packaged juices over fresh fruit juices like high calorie intake, high sugar content and depleted dietary fibres. The advertised multivitamins in packaged juices are normally synthetic and added during the processing of fruit juices. Additionally, fruit mashing, filtration, heat pasteurization and storage are expected to alter the antioxidant properties of commercially available fruit juices[9]. Some of the synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and tertbutylhydroxyquinone used as permitted preservatives in commercial beverages, when present in high concentrations, may cause genotoxicity and carcinogenicity[10]. Surprisingly, although the antioxidant activities of many fruits available in India are regularly being estimated, no effort has been made to compare the antioxidant property of packaged fruit juices with their fresh juice counterparts.

In view of this, the present study was undertaken to make a comparison between antioxidant properties of fresh and packaged fruit juices. The total phenol content (TPC) was also measured to substantiate the antioxidant capacity. To authenticate the study, packaged fruit juices of two popular brands in India were used. The study also aims to provide an insight to consumers about the impact of substituting fresh fruit juices with their commercial counterparts as a source of antioxidants.

2,4,6-Tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu reagent (FCR), gallic acid, ascorbic acid, sodium acetate, glacial acetic acid, ferric chloride and sodium carbonate were purchased from Merck, India. All the chemicals used were of analytical grade.

Fruit samples of high quality, including mango, litchi, pomegranate, orange, pineapple and apple were purchased from the local supermarket and stored in a refrigerator (5°C) until processing. The packaged juices of two popular brands were also bought from the supermarket and coded accordingly for their further usage.

The fruits were washed under running tap water and air-dried before preparation of samples. Each fruit was peeled and divided into small pieces. The fresh juices were collected by grinding these pieces and careful hand-squeezing using double-fold muslin cloth. The pomegranate was processed in two different ways. In one of the methods, only aril, including seeds was used, whereas in the other method, aril, pellicle and seeds were processed. To avoid the presence of insoluble components in the juices, the samples were diluted as required and filtered thoroughly.

Ferric ion reducing antioxidant power (FRAP) takes advantage of electron transfer reactions. Here ferric salt, $Fe(III)(TPTZ)_2Cl_3$ is used as an oxidant. The reagent was prepared freshly using the modified method of Benzie and Strain[11]. Briefly, acetate buffer (300 mM, pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ stock solutions were prepared and mixed in the ratio 10 : 1 : 1 to obtain working FRAP solution. The sample was used undiluted, as mentioned in Abountiolas and Nunes[12]. In a test tube, 0.4 ml of sample was mixed with 1.6 ml of working FRAP reagent. The tubes were further incubated at 37°C for 30 min. The optical density of the coloured ferrous tripyridyltriazine complex in the solution was measured at 590 nm. Ascorbic acid in the range 10–80 μg was used as standard. The results were expressed as mg/100 ml of ascorbic acid.

TPC was determined using modified FCR method of Singleton and Rossi[13], and Sasic-Keskin *et al.*[14]. In brief, fresh fruit and packaged juices were diluted 25 times using distilled water. Next, 0.2 ml of this diluted sample was taken in a test tube and mixed with 1 : 10 diluted FCR. The contents of the tubes were vortexed and allowed to stand for 10 min. Then, 0.8 ml of sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for 30 min. The absorbance of resulting solution was measured at 700 nm. TPC was expressed as GAE (gallic acid equivalents) in mg/100 ml of fruit sample. The concentration of polyphenols was determined from the standard curve of gallic acid in the range 2–16 μg/ml.

All the samples of fresh fruit and packed juices were run in triplicate. Standard deviation and standard error of mean were calculated and expressed in respective graphs. Analysis of variance was done using GraphPad Prism Version 5. Also, P-value < 0.0001 was considered to be significant.

Several methods are used to assess antioxidant properties of biological materials. The most commonly used

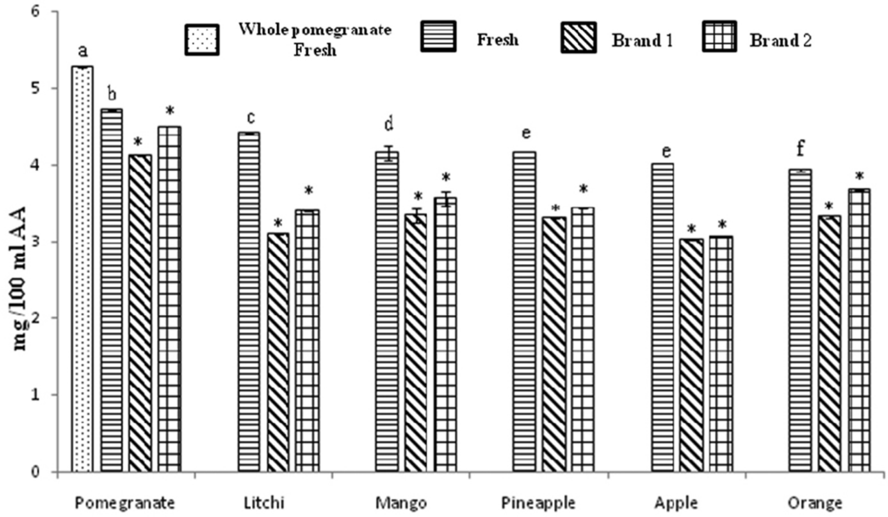

Figure 1. Ferric ion reducing antioxidant power (FRAP) values (mg/100 ml ascorbic acid) of various fresh fruit juices and packaged juices. Each value represents mean ± SEM, $n = 5$. Values with different superscripts (a–f) differ significantly at $P < 0.0001$. The FRAP values of packaged juices marked with (*) are significantly ($P < 0.001$) different compared to their fresh fruit counterparts.

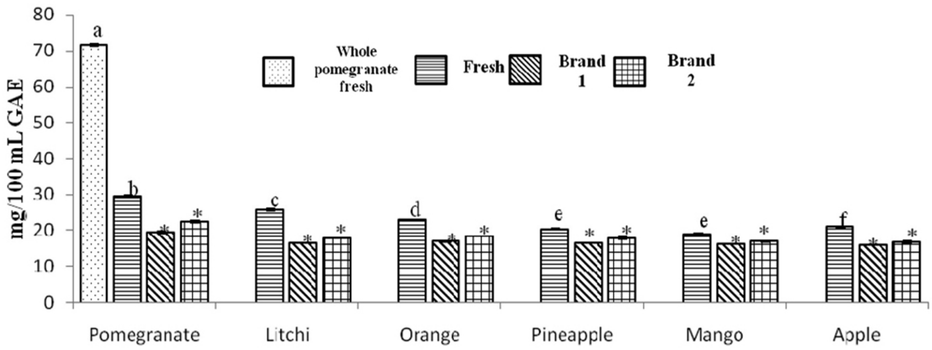

Figure 2. Total phenolic content (TPC) (mg/100 ml gallic acid equivalents) of fresh fruit juices and packaged juices. Each value represents mean ± SEM, $n = 5$. Values with different superscripts (a–f) differ significantly at $P < 0.0001$. TPC of packaged juices marked with (*) are significantly ($P < 0.001$) different compared to their fresh fruit counterparts.

methods involve the principle of conversion or disappearance of a chromogen of radical nature by antioxidants present in the analytical sample. FRAP assay was performed to measure the antioxidant activity of fruit juices. The assay measures the ability of antioxidant compounds to reduce Fe (III) to Fe (II) under acidic condition (pH 3.6). Among fresh fruit juices, pomegranate had the highest antioxidant activity (Figure 1). The order of antioxidant activity of other fruit juices was litchi > mango > pineapple > apple > orange.

Many other studies have also shown the high antioxidant capacity and phenolic content of pomegranate juice, much higher than most other fruit juices and beverages[15–17]. The prominent antioxidant compounds in pomegranate juice are hydrolysable tannins, anthocyanins and ellagic acid[15]. Due to its high antioxidant activity, pomegranate juice has been recommended as a preventive measure for coronary heart disease[18], and is also reported to augment the chemotherapeutic effects on human prostate cancer[19].

Litchi also showed very high antioxidant activity (Figure 1). Many studies have reported significant amount of polyphenols, flavonoids and anthocyanins in litchi pericarp[20] and attributed anti-inflammatory, anti-carcinogenic and immune-modulatory properties to these phenols[21–23]. In this study we have compared the antioxidant property of litchi aril that is used for making juice with other fresh fruit juices and its commercial counterpart. The variations in antioxidant activities of different types of fruit juices in the present study from those reported in the past might be due to maturity index of the selected fruits, parts of the fruits used, technique used for juice extraction, sample preparation and handling, and also exposure to oxygen or light during laboratory analysis[24–27].

Compared to packaged fruit juices of both the commercial brands, all fresh fruit juices had significantly higher antioxidant activity as estimated by FRAP assay (Figure 1). In a similar study, Wern *et al.*[28] reported that commercial fruit juices were not a good source of antioxidants compared to fresh fruit juices.

TPC of fruit juice samples was estimated using Folin–Ciocalteu assay. The assay is based on the reduction of FCR by phenolic compounds under alkaline conditions. The absorbance is directly proportional to the concentration of phenolic compounds, which is represented by the intensity of blue colour produced in each solution[29]. The highest TPC content was present in fresh pomegranate among all the fruits (Figure 2). TPC of whole pomegranate, including rind was much higher than that of fresh pomegranate juice extracted from aril only. This was also seen in the study conducted on Persian pomegranate by Shams Ardekani *et al.*[30]. In conformation with our studies, Gözlekçi *et al.*[31] also reported high TPC in peels followed by arils. TPC was found to be significantly high in fresh fruit juices compared to packaged juices, corresponding to the antioxidant activity as measured by FRAP. Similarly, higher total phenolic compounds were reported in freshly extracted fruit juices by Mahdavi *et al.*[32].

The findings from the present study suggest that fresh fruit juices are a much better source of antioxidants when compared to packaged juices. Their regular consumption can have a beneficial effect and protect us from many non-communicable diseases. Commercial juices, although easily available and attractive, could be considered as a replacement for aerated drinks but not for fresh fruit juices. Among fresh fruit juices, pomegranate has the highest total phenols and antioxidant activity. Moreover, pomegranate juice, including rind has higher antioxidant activity compared to juice extracted from arils only. Litchi, otherwise less known for its antioxidant property, can also be considered as a good source of antioxidants.

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