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Evaluation of Total Phenolics and Antioxidants of Fresh and Commercial Fruit Juices

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Abstract: **In recent scenario, rise in the life expectancy and consideration in the human health, disease prevention and nutrition, has inflated customers demand for the functional foods. As fruit juices contain constituents which are having health promoting along with antioxidant and the anti-cancer activities, they could be grouped as functional foods. This study was mainly carried out to evaluate total phenolics (TPC) and antioxidant property of the commonly obtainable fresh fruits juices (mango, apple, pomegranate and guava) and their commercial counterparts, available in popular brand found in the Indian subcontinent. TPC and antioxidant activity were evaluated by Folin-Ciocalteu assay (FC) and ferric reducing antioxidant power (FRAP) of fruit juices. Among fresh juices and packed fruit juices significantly variations ($p<0.05$) were observed. Fresh fruit juices showed higher activities than commercial juices with high antioxidants and TPC in pomegranate and low in apple respectively. This information about fresh and commercial fruit juices provide significant information mainly for the ageing population and children, for selection of healthy fruit juice for maintaining good health.**

Index Terms: **fresh and commercial juices, total phenolic content, FRAP.**

I. INTRODUCTION

Antioxidants are well-known to have valuable health promoting properties such as increasing immunity system, delaying aging process and decreasing of metabolism diseases. Intake of fruits loaded in antioxidant substances were inversely linked to risks of the non-communicable diseases (Tan, B. L et al 2018). Fruits and fruit juices contain numerous health-promoting factors including ascorbic acid, vitamins, phenolic compounds and minerals. Especially phenolic compounds have broad range health benefits like antioxidant activity, anti-mutagenic, anti-bacterial, minimize oxidative stress and anti-inflammatory (Celep & Rastmanesh 2013). The general commendation for intake of Fruits for an individual per day is of atleast 400 grams (WHO 2003, Sachdeva S.et al 2013). Similarly, national nutrition guidelines recommend average daily intake of 100 g of fruits. A glass of fruit juice each day counts as a fraction of fruit even though whole fruit is recommended for active life style (Landon S 2007).

At present, there is growing approach to supplement daily diet along with the packaged fruit juices. In India, people are witnessing a sudden change in their lifestyles and thus adopting new instant food habits. With rapid urbanization, increase in income and lack of time, consumers are more depending on packaged fruit juices than fresh fruits. Currently, consumers prefer juices with positive impact on their health. Though few of the synthetic antioxidant like tertbutylhydroxy-quinone, butylatedhydroxyanisole and butylatedhydroxytoluene were used as permitted preservatives in some commercial beverages, their presence in high concentrations, may lead to carcinogenicity and genotoxicity (Ndhlala, A. R et al 2010). Consequently, there is a need to consider the antioxidant activity and TPC, as they can effect on consumer's acceptance (Beh et al 2012).

As tropical fruits are mainly considered an important source of vitamins, phenolic, dietary fiber and antioxidants (Mitra,

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Devi, & Debnath, 2014), for this study the following fruits were selected, pomegranate, which contains important antioxidants like oleanolic, ursolic and gallic acid and helps to regulate blood sugar and prevent diabetes, [6, 7]. Mango is well known as king of all fruits, because it is rich in dietary source, carotenoids, antioxidants and phenolic compounds which have shown good health effects on the human body (Barbosa et al., 2017). Guava is remarkably considered as high antioxidant fruit due to high level of phenolic compounds (Verma et al., 2018). Apples are also abundantly rich in polyphenols, antioxidants, flavonoids and dietary fibers. The antioxidant potential of the fresh fruit juice differ from their commercial counterparts because of various reasons which include Fruit variety, extraction process, processing, clarification, filtration and pasteurization during juice production industrially. Therefore, present study was designed to compare TPC and antioxidant activities by FC and FRAP in fresh fruit juices and commercially available packaged juices of two popular brands in India and provide useful information to the consumers regarding selection of the fresh fruit juices with their commercial counterparts as a good source of antioxidants.

II. MATERIALS & METHODS

SAMPLE COLLECTION & PREPARATION

Selections of fruits and fruit juices: Four varieties of fresh fruits, namely guava, pomegranate apple and mango, were purchased from local markets in Kukatpally, JNTUH Hyderabad. Commercially available juices of two brands of all the four types were taken and labelled as Brand A & B respectively. The fruits selected were fresh and naturally ripen. Each fruit were washed under running tap water and air-dried before preparation of samples. Then fresh juice was extracted from fruits using a grater and then filtering it with a clean double-fold muslin cloth. These juices were further diluted as necessary and coded accordingly for their further usage.

CHEMICALS & REAGENTS

Gallic acid, Folin-Ciocalteu reagent, sodium carbonate, ferric chloride hexahydrate (FeCl3. 6H2O), sodium acetate, 2,4,6-tri-(2-pyridyl)-s-triozine (TPTZ), (Sigma-Aldrich, USA), glacial acetic acid, hydrochloric acid (Merck, India.). All chemicals used in this study were analytical grade.

TOTAL PHENOLIC CONTENT (TPC)

The TPC was determined by the FC method with slight modifications of Singleton et al., (1965). Juice (300 µL) was added to FC reagent (10-fold-diluted) (1.5 mL) and sodium carbonate (7.5%) (1.2 mL). Then the mixture was kept for 30 min at 37°C to react. After incubation, absorbance was taken at 760 nm by UV–Visible spectrophotometer. Gallic acid standard was used and TPC values were then expressed by mg gallic acid equivalent in liter of fruit juice (mg GAE/L).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

Antioxidant activity was measured using Benzie and Strain (1996) method by slight modifications with FRAP assay. Firstly, FRAP reagent was freshly prepared by adding 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution (2.5 ml) with 40 mM HCl,20 mM $FeCl_3$ and 0.3 M acetate buffer (pH 3.6). Briefly, 40 µL of juice was allowed to mix with 0.2 ml distilled water and 1.8 mL FRAP reagent. After incubation at room temperature for 10 min, the absorbance was taken at 593 nm by a UV–Vis spectrophotometer. Standard ferrous sulfate $FeSO_4$, $7H_2O$ was used and FRAP values were further expressed as mM Fe^{2+}/L.

STATISTICAL ANALYSIS

All the experiments were carried out in triplicates. All results were expressed with standard deviation and statistical significance at $p \leq 0.05$. Statistical analysis was done using MS Office EXCEL program me.

III. RESULTS & DISCUSSION

TOTAL PHENOLICS (TPC)

TPC are most important plant constituents which have redoxy properties responsible for the antioxidant activity. In our study, juice samples were quantified for TPC by FC assay. It is based on reducing the reagent by TPC in alkaline condition. From **Figure 1** significantly variations ($p<0.05$) were observed among TPC for fresh and the commercial fruit juices, which ranged from 84 ± 0.75 mg GAE /100 ml to 752.24 ± 2.14 mg GAE /100 ml respectively. Of all the samples that were analyzed fresh fruit juices showed higher TPC values followed by Brand A and lower TPC values were observed in Brand B. The TPC values in fresh juices were pomegranate (752.24±2.14mg GAE/100 mL), mango (512 ± 1.39 mg GAE/100 mL), guava (483 ± 0.99 mg GAE/100 mL), and apple (214 ± 0.51 mg GAE/100 mL). While the TPC values in brand A were pomegranate (634 ± 1.67 mg GAE/100 mL), mango (458 ± 1.18 mg GAE/100 mL), guava (389 ± 0.86 mg GAE/100 mL), and apple 147± 0.23 mg GAE/100 mL).and in brand B, pomegranate (412± 1.42 mg GAE/100 mL), mango (277 ± 1.38 mg GAE/100 mL), guava (211 ± 0.81 mg GAE/100 mL), and apple 84±0.13 mg GAE/100 mL).

Previously, higher total phenolic compounds were reported in freshly extracted fruit juices by Mahdavi et al. (2010). Li et al. (2015) reported values in different fruit juices ranging from 315 - 743 mg GAE/100 mL in various cultivars of China, while Mena et al. (2013) showed values for different cultivars ranging from 300 - 407 mg GAE/100 mL in Spain. The results, in this study were in accordance with previous studies. Fu et al. (2011), observed that most of the phenolic compounds in juice samples

include quercetin, kaempferol chlorogenic acid, gallic acid, luteolin, and caffeic acid. Hence in fruit juices, TPC of fruits depends on various factors like selection of variety, storage conditions and processing techniques. While processing of fruit juice, by clarification, filtration and pasteurization the TPC would strongly influence by which part of phenolic compounds might be remove which were bound to the fiber and the pectin (Candrawinata et al. 2012).

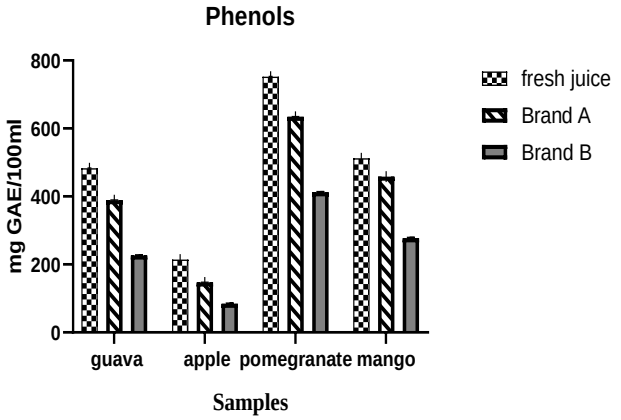

FIGURE 1. TPC (mg GAE/100 mL) of different categories of juices for each variety of fruit

FERRIC REDUCING ANTIOXIDANT POWER ASSAY

FRAP is used to measure the capability of antioxidants under acidic conditions (pH 3.6) to decrease Fe (III) to Fe (II). From Figure 2 significantly variations ($p<0.05$) were observed among FRAP values of fresh and commercial juices ranged from 79.16 ± 0.75 μmol Fe^{2+}/100 mL - 995.24 ± 1.04 μmol Fe^{2+}/100 mL respectively. Among all fruit juices, pomegranate had the highest antioxidant capacity. The order of antioxidant activity of other fruit juices were mango > guava > apple. The **Figure 2** showed higher antioxidant levels of freshly extracted juices as compared to commercially available juices. Brand B of all fruit juices, showed lower levels of antioxidant capacity as compared to Brand A and fresh juices. High levels in fresh juices were evident as they were having higher amounts of antioxidants.

Earlier studies by Nuncio Jauregui, N et al (2015) and Gill, M. I. et al (2000) have shown that high antioxidant capacity and phenolic content of pomegranate juice, were much higher than most other fruit juices. The important antioxidants present in pomegranate juice are ellagic acid, anthocyanins and tannins. According to Densupsoontorn N et al (2000) commercially available fruit juices undergo various at industrial processes like squeezing, pasteurization, and freezing may affects antioxidant levels. These variations in the antioxidant activities of different fruit juices in the present study might be because of selected fruits, procedure used for juice extraction, handling and sample preparation, in laboratory analysis (Sreekumar, S, et al 2014).

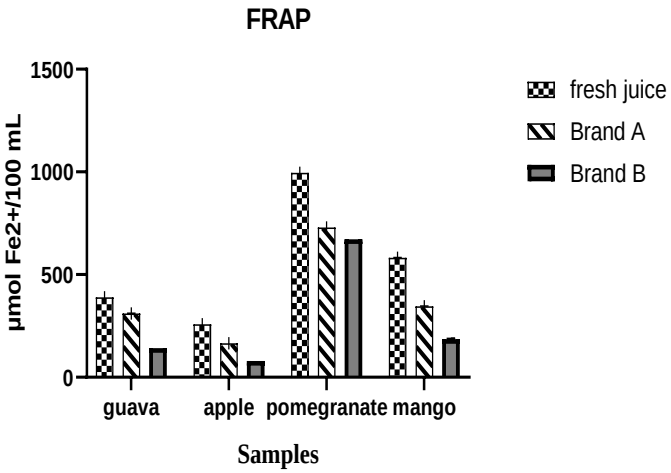

FIGURE 2. FRAP values (μmol Fe^{2+}/100 mL) of different categories of juices for each variety of fruit.

CONCLUSION

The TPC and antioxidant levels of freshly extracted fruit juices and commercially available fruit juices of two brands were measured by performing Folin-Ciocalteu assay and FRAP assay. It was accomplished that pomegranate provided highest antioxidants as compared to that of other fruits. It was also observed that fresh fruits always serve high levels of antioxidants as compared to commercially available juices. Hence from the present study fresh fruit juices especially pomegranate can be considered as a good source of antioxidants. Commercially available juices undergo various processing due to which the antioxidants maybe lost. So it is always recommended to consume fresh fruit juices rather than commercially fruit juices and further the enhancement of commercial fruit juices can be achieved by increasing the antioxidant levels from natural sources. Therefore the food industry can focus on development of new functional foods and beverages with added health benefits.

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