Comparative Studies on Phytochemical Composition and Antioxidant Capacity of Fresh and Commercialized Apple Juices

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Abstract**:** Apples are nature's marvelous gift to the humankind, indeed, they are life-enhancing medicines enriched with phenols, vitamins, minerals, antioxidants and many phytonutrients. Due to the increased awareness of healthy lifestyle, people normally rely on apple juices. Because of the busy life schedule, they depend on the commercialized/carbonated apple juices. The present study was undertaken to detect the actual amount of phytochemicals and antioxidants in Apple Fresh Juice (A.F.J), Apple Commercialized Juice (A.C.J), and Apple Commercialized Carbonated Juice (A.C.C.J). The determination of phytochemicals, analysis of antioxidants, scavenging activity and metal chelating activity by ABTS, DPPH, FRAP was undertaken in the present study. The fresh juice has rich vitamin C than that of commercialized juices and this indicates that the amount of Vitamin C would have been reduced during the processing of juices. The commercialized juices have rich antioxidants than that of fresh juices it indicates that the antioxidants are added by the beverage industries with preservatives while processing the beverages. Since the juices are pure & fresh and devoid of preservatives, it does not allow the microorganisms to grow. Also the acidic condition is maintained to inhibit the microbial growth and shelf life was analyzed by plating technique. From the study, it was observed that, consuming fresh fruits or fresh fruits juices are safe, effective and healthy as they are enriched with rich nutrients compared to the commercialized /carbonated juices.

Keywords: Apples, Phenols, Vitamins, Minerals, Antioxidants, Phytonutrients, ABTS, FRAP, DPPH, Fresh and Commercialized Juices.

I. INTRODUCTION

Apples are widely consumed fruit throughout the world, and a rich source of phytochemicals, and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes [1]. In 2011, world apple production was estimated to be around 75 millions of tons according to Food and Agriculture Organization states (FAO) [2]. The famous sentence: "*An apple a day keeps the doctor away*!" is what is highly recommended and heavily advertised nowadays to the general public to stay fit and healthy [1]. Apple's fruit, as well apple juice and other derivate products, have a rich phytochemical profile suggesting their potential to have a good impact among the populations consuming them [3].

A major role of the phytochemicals is protection against oxidation. We live in a highly oxidative environment and many processes involved in metabolism may result in the production of more oxidants [4]. In the United States, twenty-two percent of the phenolics consumed from fruits are from apples making them the largest source of phenolics [5]. Apples were one of the main sources of dietary flavonoids that showed the strongest associations with decreased mortality [6]. The health-promoting effect of antioxidants from apple is thought to arise from their protective effects by counteracting reactive oxygen species [7]. The compounds that assist restrain and defer lipid oxidation, when added to nourishments will in general limit rancidity, hinder the arrangement of poisonous oxidation items, help keep up the wholesome quality and increment their timeframe of realistic usability are called as antioxidants [8].

Quality of fresh apple juices and puree can be affected by different factors like cultivar, geographical region, climate, cultivar practices, harvest [9], storage conditions [10], and processing [11], [12], [13]. The incorporation of apples and their derivatives in the diet mainly for their antioxidant properties, associated to the polyphenols and vitamin C content, is considered useful since these compounds can contribute to reduce the risk of coronary heart disease, carcinogenesis, aging processes, and can inhibit human low density lipoprotein oxidation [14], [15], [16]. Purchasers demonstrate an expanding enthusiasm for organic product juices with a high normal cell reinforcement's content, for example nutrients and polyphenols [17].

Apples have been found to have very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol [18]. The parameters considered relevant to describe the overall quality of fruit juices and their processed derivatives are the reducing sugars, vitamin C content, phytochemicals, and the total antioxidant activity. On the other hand, the processing of beverages, which is generally used to extend shelf-life of fruit products, can also affect these parameter's descriptors of the quality [17]. The most important transformations during the processing is the concern for the loss of phytochemical content and changes in antioxidant compounds, non-enzymatic browning reactions [18], and the formation of undesirable products like 5-hydroxy methylurfural (5-HMF). Various analytical methods such as DPPH, FRAP, ABTS have been reported to evaluate the real amount of antioxidant bio-available compounds in juices and puree, and an ***in vitro*** digestion procedure is very much needed to check the quality indeed. [19], [10], [20].

Hence present work was undertaken to compare the fresh apple juice and commercialized apple juices with and without carbonated conditions, based on their phytochemical content, antioxidant activity, scavenging activity, metal chelating activity and its shelf- life.

II. MATERIALS AND METHODS

2.1 Plant Materials

Fresh apples were purchased from local market of Chennai (Pazhamudircholai). The procured fruits were washed and dried at room temperature. The outer portion of these fruits was wiped with 70% alcohol and cut into small pieces to remove the seeds. The fresh juice was collected by using the sterile juicer. The well-known commercialized apple juices were brought from the local supermarket.

2.2 Phytochemicals Assessment of Juices

The qualitative analysis of fresh and commercialized apple juices was carried out to determine the presence of proteins, carbohydrates, phenols and tannins, saponins, flavonoids, glycosides, sugar, steroid [21].

The quantitative analysis of fresh and commercialized apple juices was carried to determine the amount of soluble carbohydrates, sugar, flavonoids phenols and tannin [22].

2.3 Determination of Vitamin C

The Vitamin C content of the fresh and commercialized apple juices were determined by the methods described by Erhabor et al 2015 [23].

2.4 Ferric-Reducing/Antioxidant Power (FRAP) Assay

The antioxidant capacity of the fresh and commercialized apple juices were estimated according to the method described previously by Pulido et al.,2000) [24]. The absorbance of the reaction mixture was read at 593 nm. The free radical scavenging activity was expressed as Milligram of catechin equivalent per Gram of sample.

2.5 Antioxidant Activity by The ABTS+ Assay

Radical scavenging activity of the fresh and commercialized apple juices were assessed spectrophotometrically by [2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] ABTS+ cation decolonization assay and the absorbance was taken at 734nm [25]. Results were expressed as milligrams of catechin equivalent per gram of sample [26].

2.6 Metal Chelating Activity

The chelating activity of ferrous ions in the fresh and commercialized apple juices were estimated by the method described by Dinis et al. (1994) [27]. Absorbance of the solution was measured spectrophotometrically at 562 nm. The results were expressed as mg ethylene diamine tetra acetic acid (EDTA) equivalent/g extract [28].

2.7 Free Radical Scavenging Activity on DPPH

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity of the fresh and commercialized apple juices were measured according to the method of Kedare and Sing 2011 [29]. The antioxidant content and inhibition rates of DPPH were calculated as milligram of catechin equivalent per gram of sample with 517nm absorbance [24]. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity [30]. The free radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity.

2.8 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the fresh and commercialized apple juices were determined [31]. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The hydroxyl radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity [32].

2.9 Purity Analysis of Juices

The purity level of the juices was checked by the microbial growth in plating technique using E. coli, Yeast and Mould, Total Plate Count, Coliform.

III. RESULT AND DISCUSSION

3.1 Phytochemicals Assessment of Juices

The phytochemical analysis showed that the carbohydrates, sugar, flavonoid, phenol, tannin and vitamin C are present in fresh apple juice as well as in carbonated and decarbonated commercialized apple juices (Table 1) and its quantitative analysis (Table 2).

Table 1
Qualitative Phytochemical Test (+: Present, -: Absent)

Phytochemicals	Carbohydrates	Proteins	Phenols and tannins	Flavonoids	Saponins	Glycosides	Steroids	Sugar
A.F.J	+	-	+	+	-	-	-	+
A.C.J	+	-	+	+	-	-	-	+
A.C.C.J	+	-	+	+	-	-	-	+

Table 2
Quantitative Test for Phytochemicals (mg/100ml)

JUICES	CARBOHYDRATE $\times 10^3$	SUGAR $\times 10^3$	FLAVANOID	PHENOL	TANNIN	VITAMIN C
A.F.J	16.5	3.5	49.7	81.5	64.26	4.85
A.C.J	15.8	15.8	48.2	80.2	55.15	3.38
A.C.C.J	13.6	15.8	49.2	79.1	51.12	3.21

3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Antioxidants can be explained as reductants, and in activators of oxidants [33]. Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be related to reducing power. Therefore, the antioxidant potential of all the three juices was estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II). The ferric reducing ability of the juices revealed that all of them have good FRAP activity (714.8–820.6 mmol Fe (II)/g sample). Among the juices, the highest activity was noted for A.C.C.J (820.6 mmol Fe (II)/g sample) followed by A.C.J (721.2mmol Fe (II)/g sample) The A.F.J had lower FRAP activity (714.8 mmol Fe (II)/g extract). Halvorsen et al. (2006) suggested most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP Assay [34].

3.3 Antioxidant Activity by the ABTS+ Assay

Apple juices are fast and effective scavengers of the ABTS radical. In ABTS+ scavenging activity the values are ranged from to 54538.2 to 58351.6 mlmol Trolox/ml sample). Among the juices, the highest activity was noted for A.C.J (58351.6 mlmol Trolox/ml sample) followed by A.C.C.J (55471.2 mlmol Trolox/ml sample.) The A.F.J had lower ABTS activity 54538.2 mlmol Trolox/ml sample.). Actually, the ABTS radical cation scavenging activity also reflects hydrogen-donating ability. Hagerman et al. (1998) reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS+) [35]. Since, the extracts from various samples have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction, they could serve as potential nutraceuticals when ingested along with the nutrient.

3.4 Metal Chelating Activity

Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress [36]. An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton type reactions. Therefore, it is considered important to screen the iron (II) chelating ability of the extracts. The juices demonstrated a moderate level of ability to chelate metal ions as 2.6 to 3.2 mg EDTA/ ml extract. Among the juices, the highest activity was noted for M.F.J (3.2 mg EDTA/ ml extract) followed by A.C.J (2.7 mg EDTA/ ml extract) The A.C.C.J had lower chelating activity (2.6 mg EDTA/ ml extract). The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups [37].

3.5 Free Radical Scavenging Activity on DPPH

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant [38]. It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts scavenging [39]. The scavenging abilities of apple juice were concentration-dependent and expressed as IC50 values. It is necessary for the concentration of the sample to decrease the initial concentration of DPPH by 50%. (IC50) under the experimental condition was calculated. Therefore, a lower IC50 value indicates a higher antioxidant activity. Among the juices, the highest activity was noted for A.F.J (38%) followed by A.C.J (34%). The A.C.C.J had lower activity (32%).

3.6 Hydroxyl Radical (OH) Scavenging Activity

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2 which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage in vivo [40]. Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides [41]. Thus, removing OH is very important for the protection of living systems. The hydroxyl radical scavenging potential of juices was found to be 65.7 to 78.9% which is actually a good scavenging effect. Among the juices, the highest activity was noted for A.F.J (78.9%) followed by A.C.J (74.2%). The A.C.C.J had lower activity (65.7%).

3.7 Purity Analysis of Juices

The fresh apple juice (A.F. J), commercialized apple juice (A.C. J) and commercialized carbonated apple juice (A.C. CJ) were tested by plating method using different source such as Total Plate Count (T.P.C), Yeast and Mould plate count(YM), Coliform plate count and *E. coli* plate count.

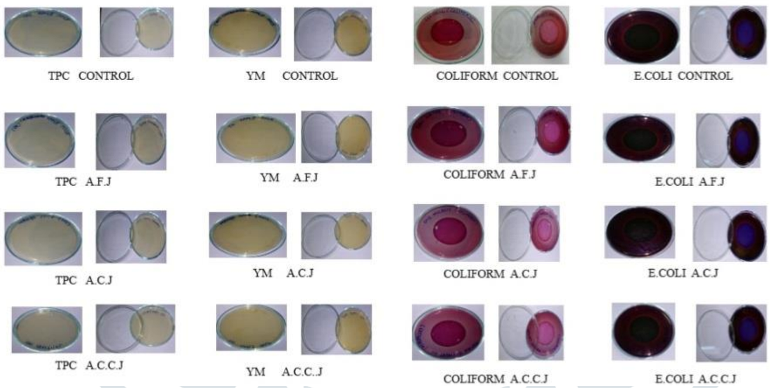

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Figure 1

Testing of Microbial Growth by Plating Method

Figure 1 shows that there is no microbial contamination in any of the juices. The sample would be rejected if any microbial growth is found.

IV. CONCLUSION

The phytochemicals such as carbohydrates, phenols, tannins, flavonoids and sugars are present qualitatively and in the quantitative analyses commercialized juices contained a higher amount of carbohydrates and sugar than that of fresh fruit juices. It indicates the addition of reduced sugar while processing. Fresh juices had high content of Tannins and Vitamin C than the commercialized juices. There is not much difference in the flavonoid and phenol content, so it is understood that the phenol and flavonoid content are not affected while processing.

FRAP and ABTS assay shows that the commercialized juices had high antioxidant capacity than the fresh juices. It indicates that the antioxidants are added by the commercialized industries with preservatives while processing of juices. Fresh apple juices had rich scavenging and metal chelating activity than commercialized juices; it indicates that the scavenging and chelating properties of juices were reduced while processing of juices. Apple juices are strong radical scavengers and can be seen as potential source of natural antioxidants for medicinal and commercial uses.

Regular consumption of apples, as part of a healthy diet may aid in the prevention of chronic disease and maintenance of good health. In conclusion, our results suggest that there are no significant changes in all the values of fresh and commercialized juices. There is no microbial growth in both fresh and commercialized juices which indicates the good purity level of the beverages. Consuming whether fresh fruits or fresh fruits juices or commercialized fruits juices does not matter. It should be safe, effective and healthy. Promising brands of commercial juices would be recommended. All such kind of novel research is beneficial to common man in order to have awareness about the genuine conditions of our consumables and to stay safe & healthy

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