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Comparative Analysis of Ascorbic Acid Content and Antioxidant Activity of Some Fruit Juices in Syria

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ABSTRACT:

Fruits are considered essential constituents of a healthy diet. They possess antioxidant properties which can be attributed to the presence of antioxidant compounds. Ascorbic Acid (AA) is a water soluble antioxidant, abundant in fresh fruits and juices and considered as one of the most important contributors to the total antioxidant activity of fruits and vegetables. Two samples of the following fruit juices (orange, grapefruit, black mulberry, strawberry, pomegranate and tomato) were investigated for their AA content and total antioxidant activity. AA content was determined according to dichlorophenol indophenol method. Orange juice showed the highest results with amounts from 74.85mg/100 ml to 101.28mg/100 ml, while the lowest was pomegranate juice with amounts from 8.6mg/100 ml to 8.8mg/100 ml. Total antioxidant activity was subsequently determined using Ferric Reducing Antioxidant Power (FRAP) assay and reducing power assay. The results indicated that black mulberry juice had the highest antioxidant power, while tomato juice had the lowest. The study concluded that AA plays a dominant role as an antioxidant only in fruit juices which are known to be rich in AA. Other juices showed higher antioxidant activity which indicated the role of other antioxidant compounds i.e. phenolic compounds.

KEYWORDS: Ascorbic acid, antioxidant activity, Reducing Power.

INTRODUCTION:

Fruits play a positive role in reducing the risk of chronic diseases such as cardiovascular diseases, some types of cancer[1,2,3], aging[4]and obesity[5]. Epidemiological evidence suggests a negative association between fruit consumption and the incidence of several chronic diseases[6]. Thus, high intake of fruits is recommended[1,7]. The absence of fat and the high amounts of sugar together with vitamins make fruits and their juices of great help in liver diseases.

On the other hand, fruit juices are valuable therapeutic agents in acute and chronic kidney diseases due to their low content of nitrogen. Fruits and vegetables also inhibit the formation of bile and kidney stones since they are high in potassium salts, thus they are recommended in treatment of acidosis, diabetes, under-nutrition, gout and aging tissues [4]. Fruits also play a positive role in gastrointestinal disorders, central nervous system (CNS), dermatological diseases, metabolic disorders and respiratory diseases[5].

These health effects can be attributed to the presence of vitamins such as A, E and AA, dietary fibers and non-essential phytochemicals[2] such as phenolic compounds[7]. They are also rich in carotenoids, glutathione and antioxidant enzymes[4]. Most of those compounds are free radical scavengers which inhibit lipid peroxidation and reactive oxygen species (ROS) formation to maintain small amounts required for normal cell function[6]. Recent research also suggests that antioxidants present in most fruit juices can lower the risk of Alzheimer's disease[4].

AA is one of the phytonutrients essential for cell metabolism[7]. AA is a water soluble antioxidant, abundant in fresh fruits and juices[8,9,10]. It is a co-factor in several enzymatic reactions such as those incorporated in the synthesis of collagen[9,10,11] as well as carnitin[8]. These reactions are essential in wound healing and in preventing bleeding from capillaries[11]as well as bone formation and scar tissue repair[8,9]. AA deficiency leads to scurvy[8,11]which causes bleeding from mucosal tissues as well as spongy gum and skin pigmentation. For that reason, it is used in supplements and fortified orange juice[9]. AA also plays a role in several physiological processes leading to beneficial effects on immune response, cancer and cardiovascular diseases[12]. It can lower blood pressure and cholesterol levels, mitigate the severity of cold and prevents viral or bacterial secondary infection. AA lowers the risk of several types of cancer including breast, cervix, colon, rectum, lungs, prostate and stomach cancer[8].

AA can be found in different amounts in natural foodstuff especially fruits and their products. AA is one of the most important contributors to the total antioxidant activity of fruits and vegetables[7]. In fact, it is considered one of the most powerful natural antioxidants[4].Therefore, it is used to prevent oxidative damage in food[11]. It works as a reducing agent and free radical scavenger for superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals since it is an electron donor[9]. It can also maintain the redox status inside the cells by keeping sulfhydryl compounds including glutathione in their reduced state[13].

Since Syria is very rich in fruit cultivars, it is important to assess the AA content in several different juices especially that there is a lack of studies in Syria in this area. It is also essential to study the correlation between AA content and the antioxidant activity since most studies focus on the role of phenolic compounds in the antioxidant activity. So, the aim of this study is to determine AA levels in several frequently consumed juices in Syria and to assess AA contribution to the total antioxidant activity of these juices.

MATERIALS AND METHODS:

Chemicals and Apparatus:

2,6-dichlorophenolindophenol Sodium salt Dihydrate (DCPIP) and 2,4,6-Tris(2-pyridyl)-s-triazine were purchased from Fluka, Germany. Sodium Hydrogen Carbonate was purchased from Qualikems, India. Di Sodium Phosphate was purchased from Merck, Germany. Ferric Chloride anhydrous was purchased from Qualikems, India. Ferrous sulfate was purchased from BDH, England. Potassium ferricyanide was purchased from May and Baker LTD, England. Sodium Acetate was purchased from BDH, England. Sodium Phosphate was purchased from Riedel-De Haen AG, Germany. Trichloroacetic acid was purchased from Riedel-De Haen AG, Germany. Analytical balance (RADWAG, AS 220/C/2). Micropipette was purchased from Labkit (Chemelex, S.A., Spain). Spectrophotometer (Jasco V-530 UV). Waterbath, Water bath (K and H Industries). Oven (CARBOLITE).

Samples:

Two samples of each of the following fruits were purchased from local markets: orange, grapefruit, black mulberry, strawberry, pomegranate and tomato. Juice samples were prepared according to Dumbravă*et al*. (2011). Each fruit was washed, peeled, squeezed then filtered and kept in the freezer (-20°C) until analysis. All samples were diluted properly in distilled water prior to analysis.

Ascorbic Acid Content:

The AA content was determined in each juice using DCPIP solution[14]. Five ml of the properly diluted sample was titrated with DCPIP solution (0.001 N) until a pale pink color is observed.

Ferric Reducing Antioxidant Power:

The Ferric Reducing Antioxidant Power (FRAP) was measured spectrophotometrically[15]. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mMHCl and 20 mM ferric chloride solution at a ratio of 10:1:1 (v/v/v) respectively. 1 ml of FRAP reagent was added to 100 µl of juice sample. The mixture was then incubated for 4 min at room temperature. Absorbance was measured at 593 nm and the results were calculated according to a calibration curve obtained using $FeSO_4$ solutions in concentrations between (150-500 µM). The results were

expressed as mM Fe^{2+}/L juice.

Reducing Power:
The reducing power of the diluted sample was determined spectrophotometrically[16]. 0.5 ml of the diluted juice sample were mixed with 0.5 ml of phosphate buffer 0.2 M, pH 6.6 and 0.5 ml of 1% potassium ferricyanide solution. The mixture was incubated at 50ºC for 20 min in a water bath then 0.5 ml of 10% (w/v) trichloroacetic acid were added to the mixture. Finally, 1 ml of the mixture was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride solution. Absorbance was measured at 700 nm. Increased absorbance indicated increased reducing power. Since each juice had a different dilution ratio, it was not possible to compare the absorbance directly. Due to the similarity between this assay and FRAP assay, a calibration curve was prepared using $FeSO_4$ solutions in concentrations between (250-750 µM). The results were expressed as mM Fe^{2+}/L juice[17].

Statistical Analysis:
All data were reported as mean ± standard deviation of duplicate determination. The differences between FRAP assay and the reducing power assay were determined by Student's t-test.

RESULTS AND DISCUSSION:

Ascorbic Acid Content:
AA results are displayed in table 1. Orange juice had the highest AA content (74.85 and 101.28 mg/100 ml). These results were higher than those obtained by several other studies. For example, Nweze et al. (2015) results were as low as 10.13 mg/100ml[11]. However, according to Ywassaki and Canniatti-Brazaca (2011), AA content in orange juice was 42.35 mg/100 g[18]. Similarly, Igwe's (2014) results were 49.50 mg/100 g[19]. These differences in results can be attributed to several factors including climate, sun exposure, species, ripening, storage conditions among others[19].

Table 1: Ascorbic acid concentration, FRAP and Reducing Power results

Juice type	Sample number	Ascorbic acid concentration		FRAP (mM Fe^{+2}/L_{juice})	Reducing Power (mM Fe^{+2}/L_{juice})
		(mg/100ml)	(mg/100g)		
Orange	1	74.85 ± 0.6	71.41 ± 0.6	12.45 ± 0.5	9.37 ± 0.1
Orange	2	101.28 ± 0.6	96.62 ± 0.6	8.51 ± 0.3	4.63 ± 0.2
Grapefruit	1	57.24 ± 0.6	55.35 ± 0.6	11.51 ± 1.1	8.87 ± 0.2
Grapefruit	2	70.45 ± 0.6	68.13 ± 0.6	7.76 ± 0.5	5.05 ± 0.1
Tomato	1	33.02 ± 0.3	32.09 ± 0.3	3.92 ± 0.1	3.69 ± 0.2
Tomato	2	22.01 ± 0.2	21.39 ± 0.2	5.79 ± 0.3	4.81 ± 0.1
Strawberry	1	17.61 ± 0.1	17.31 ± 0.1	23.61 ± 1.6	21.19 ± 1.8
Strawberry	2	17.35 ± 0.4	17.06 ± 0.4	20.30 ± 0.9	17.45 ± 1.3
Mulberry	1	13.21 ± 0.6	12.34 ± 0.6	66.23 ± 0.3	57.52 ± 1.7
Mulberry	2	17.12 ± 0.1	15.99 ± 0.1	59.98 ± 0.2	42.52 ± 1.5
Pomegranate	1	8.8 ± 0.2	8.33 ± 0.2	25.87 ± 0.8	22.97 ± 0.2
Pomegranate	2	8.6 ± 0.1	8.14 ± 0.1	27.12 ± 1.4	33.22 ± 0.3

Grapefruit juice content of AA was (57.24 and 70.45 mg/100 ml). Khosravi and Asadollahzadeh (2014) and Tareen et al., (2015) arrived at considerably lower results, 8.9 mg/100 ml[20]and 10.9 mg/100 ml[21], respectively. Pisoschi et al. (2011) and Shrestha et al. (2016) found that AA content in grapefruit juice was 35.23 mg/100 ml[10] and 37.5 mg/100 ml[22], respectively. Our results were in accordance with Wu et al. (2007) which were 97.9 mg/100 ml[23]. In contrast, Al-Musharfi et al. (2015) found that grapefruit content of AA was 709 mg/100 ml[24].

Several authors explained the variance in AA content in grapefruit juice by the effect of environmental factors and geographical location[24], humidity, temperature, physical damage and other postharvest conditions which may lead to a decrease in AA content[25].

It is important to mention that the beneficial effects of consuming citrus fruit juice, such as their antioxidant and anti-inflammatory potential and their protective role against heart diseases, are correlated with the presence of several bioactive ingredients like AA[20].

Even though tomato juice content of AA was lower than citrus fruits, it is considered as a rich source of AA, providing 40% of the daily value[26]. Tomato has showed to help in preventing certain kinds of cancer such as breast cancer[27]. Our results (22.01 mg/100 ml and 33.02 mg/100 ml) were close to other studies. For example, Pisoschi et al. (2011) arrived at similar concentrations of AA in tomato juice (18.92 mg/100 ml)[10], while another study has found AA concentration to be 21.14 mg/100 g[27].

Strawberry is considered as a good source of AA[28]. The present study showed that strawberry juice content of AA was 17.35 and 17.61 mg/100 ml. Several studies in the literature reported considerably higher results: 44-60 mg/100 g[29], 41.2 mg/100 g[28], 40-85 mg/100 g[30]. This can be explained by the susceptibility of AA to oxidation during storage[31]. Nevertheless, AA content in strawberry can change with cultivar, growing and storage conditions[28].

Mulberry juice has antioxidant properties as it is a rich source of phenolic compounds[32]and has moderate concentration of AA[33]. Our results indicated that the AA content of black mulberry juice was 13.21 and 17.12 mg/100 ml. Other studies reported different results. According to Ercisli et al. (2010), AA content was 20.79 mg/100 ml[34]. Khalid et al. (2011) also arrived at a similar number (23.45 mg/100 ml)[32].

While Akin et al. (2016) found AA content to be 13 mg/100g[35]. On the other hand, Eyduran et al. (2015) results were between 10-16 mg/100 g[33]. Black mulberry has been used for its protective role toward the liver and joints[36] as well as its antimicrobial and anti-inflammatory properties[32].
Pomegranate juice content of AA was the lowest among the studied juices as the results were 8.6 and 8.8 mg/100 ml. These results were close to those reported by Akhtar et al. (2013) which was 8 mg/100 ml[37]. However, some other studies reported higher concentrations as follows: 9.68-17.45 mg/100 ml[38], 23.15 mg/100 g[39] and 57.8 mg/100 g[40]. On the other hand, some studies found lower concentrations: 6.1 mg/100g[41] and 2.77-9.48 mg/100 ml[42].

Fruit juice is a major source of AA. However, several factors may alter its content in the type of fruit itself. These factors include species, growing conditions, sun exposure[43], climate, stage of ripeness, container, handling, storage and others[9]. Additionally, AA has low stability and may be oxidized with elevated temperature and pH[43].It may also decompose easily in aerobic and anaerobic conditions depending on oxygen, exposure to light, temperature and duration of storage[9]. All the previous factors can affect the content of AA in fruits.

Antioxidant Activity:
Antioxidant activity is known to be correlated with the antioxidant composition of fruits, so high amounts of antioxidants contained in the fruit will result in high antioxidant activity[40].

The antioxidant activity of the studied juices according to FRAP method and the reducing power assay are shown in table 1.

Orange, grapefruit and tomato juices had the highest content of AA among the studies juices (table 1). However, they possessed the lowest antioxidant activity. This indicates that AA is not the only component contributing to the antioxidant activity[24].

Strawberry juice possessed a relatively high antioxidant activity which was higher than those of orange and grapefruit, despite that its content of AA was lower. Van De Velde et al. (2013) stated that AA is responsible for 15% to 30% of the total antioxidant activity while anthocyanins and ellagitannins are the major contributors[28]. Stolfa et al. (2014) also reported that there's a direct relationship between the antioxidant activity and the total phenolic content[31] which was also proved by one of our previous works[17].

Black mulberry juice proved its effectiveness in scavenging superoxide, hydroxyl and nitric acid[32]. Even though black mulberry juice contained only a moderate amount of AA, it possessed the highest antioxidant activity in both methods (table 1). This can be explained by the high concentration of anthocyanins, flavonoids and other phenolic compounds which possess a high antioxidant activity[44]. It is worthy to mention that one of our previous studies on fruit juices showed a significant relationship between phenolic compounds and antioxidant activity. Black mulberry juice after elimination of AA still possessed the highest antioxidant activity compared to grapefruit, orange, tomato and other fruit juices which indicates that its content of phenolic compounds is responsible for the antioxidant activity[45].

Pomegranate juice had the lowest concentration of AA among the studied juices. However, it had nearly the second highest antioxidant activity and was advanced only by black mulberry. This finding was explained in literature by its phenolic content which are considered as potent antioxidants[39].

No correlation was found between AA concentration and the antioxidant activity using both FRAP (Fig 1) and the reducing power assay (Fig 2). Al-Musharfi et al. (2015) arrived at similar conclusion, stating that phenolic compounds in fruit juices also contribute to the antioxidant activity along with AA[24].

Figure 1: correlation between AA concentration and FRAP assay

However, there was a strong correlation between AA concentration and the antioxidant activity only in grapefruit, orange and tomato which are considered some of AA richest sources though it contributes greatly to the antioxidant activity.

This result can be coupled by our previous results which indicated a low phenolic content in these three fruit juices which minimizes the phenolic contribution to the total antioxidant activity, making AA the major contributor[17].

This means that the correlation between AA and the antioxidant activity is clear only when AA contributes strongly to this activity, and when the phenolic content is relatively lower.

Figure 2: correlation between AA concentration and the reducing power assay

Reducing Power versus FRAP:
In the last part of our study, we wanted to know if the Reducing Power assay can be used instead of FRAP since the Reducing Power assay is relatively cheaper and more convenient in some research. In most studies, the Reducing Power assay depends solely on absorbance to compare the antioxidant activity of different samples.

Due to the similarity between FRAP method and reducing power method we have prepared a calibration curveusing ferrous sulfate according to our previous study. Calibration curve allows the comparison of the studied juices since each juice was diluted differently which eliminated the possibility of comparing absorbance directly[17].

Statistical analysis using Students' T test showed significant differences between the two methods. However, due to the similarities in correlation coefficients, the reducing power assay may serve as a substitute of FRAP assay in some pilot studies.

CONCLUSION:
In the present study AA content was correlated with the antioxidant activity only in three fruit juices (orange, grapefruit and tomato) while no correlation was found in the other juices (black mulberry, strawberry and pomegranate) which possessed higher antioxidant activity even though their AA content was lower. These results indicate that the antioxidant activity of AA is only pronounced when it is present in high amounts accompanied by low concentrations of other antioxidant compounds.

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