COMPARITIVE BIOCHEMICAL ANALYSIS OF FRUIT EXTRACTS OF VARIOUS CITRUS VARIETIES AND TETRA PACK ORANGE JUICE

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Abstract

In present study, the biochemical content of citrus varieties commonly known as kinnow, mausami, grapefruit and lemon was investigated, in which titratable acidity, reducing sugar content, ascorbic acid content, total soluble solids (TSS) and antioxidant activities were estimated. The biochemical analysis was then compared with tetra pack orange juice. Grapefruit juice contained highest content 0.9 g of ascorbic acid while tetra pack juice contained lowest ascorbic acid content 0.3 g. Grapefruit juice contained highest percentage of titratable acidity 0.7 %, titratable acidity was assessed by acid base titration. TSS of kinnow and tetra pack juice was 10 %, whereas mausami contained Brix of 9 (9 % of TSS). Grapefruit had highest percentage of antioxidant activity i.e., 81 %. The reducing sugar content was measured by Benedict reagent; mausami has more than 2 %, whereas tetra pack does not contain any reducing sugar. Grapefruit juice had maximum amount of Vitamin C, titratable acidity and antioxidants which further enhanced its importance as antiaging and in body fat reduction.

Keywords: Biochemical analysis, tetra pack juice, TSS, DPPH assay, ANOVA.

Introduction

The genus Citrus constitutes of around forty species, the most conspicuous genus of family *Rutaceae* widely distributed in Australia, China, India, Srilanka and Malaysia. Its nutritional value and peculiar flavor have made this genus renowned around the globe and utilized as fresh fruit or as source of fresh juice. The health-related benefits of citrus fruits are due to various reported bioactive compounds, such as carotenoids and vitamin C (Ghafar *et al*., 2010).

Pakistan is the fifth largest citrus exporter in world. Kinnow (mandrain =*Citrus reticulata*) is the most important fruit crop of Punjab and contributes a lion share i.e. 95 % in Pakistani exports. At present, total area under citrus cultivation has recorded around two hundred thousand hectares and production is around eighteen hundred thousand tones. Mandrain is admirable because of its flavor and taste in addition to its nutritional importance. Moreover, an average orange fruit yields sixty calories along with rich source of vitamins (A, B1and B6), calcium, potassium magnesium and iron (Ghani *et al*., 2017).

Citrus fruits such as orange, lemon and grapefruit share the same fruit development, morphology and maturation besides the percentage constitution of sugars and vitamin C (ascorbic acid). Ascorbic acid is water-soluble, white, and crystalline, vitamin when pure and highly abundant vitamin in citrus fruits (Igwe *et al*., 2014).

Global orange production for 2018/19 expanded upto 4.2 million metric tons from the previous year to 51.8 million tons as weather was favorable which led to larger crops (USDA, 2019). Citrus juice consumption is found to be helpful in averting chronic asthma and coronary diseases. Citrus fruit extracts are also reported to have anti-inflammatory, antioxidant, antifungal, anti-tumor, blood anti-coagulation and anti-fungal properties (Ghafar *et al*., 2009). Vitamin C and phenolic compounds are important antioxidants present in orange juice (Wang *et al*., 1996)

The reactive oxygen species produced in body can cause oxidative damage to several cell components and may cause various ailments. The inflicted damage may contribute to aging and to degenerative diseases such as brain dysfunction, cataracts, cancers, and cardiovascular diseases (Ko *et al*., 1998). Therefore, natural antioxidants present in orange juice can neutralize free radicals, due to their ability to act as free radical scavengers (Wang *et al*., 1996).

In the presented work various orange extracts were screened especially to compare the vitamin C content and antioxidant ability which were detected in the fresh extracts while the tetra pack extract was only containing high sugar content which makes it unhealthy for health.

Materials and Methods

Plant Samples: Four locally produced citrus fruits in Punjab (kinnow, mausami, lemon and grapefruit) as fresh juice source and regular orange juice in tetra packs as preserved juice source, were purchased from

commercial market in Lahore. The average weight of each fruit was 500–650 g.

Estimation of ascorbic acid content in fruit juice: Juice was extracted and filtered through muslin cloth. Twenty ml of the sieved fresh juice sample was pipette out in a conical flask. Ten ml of distilled water was added to it and titrated with 0.005 mol/L iodine solution using 2 mL starch as indicator till the appearance of blue-black color due to the starch-iodine complex and then the amount of ascorbic acid was calculated (Nweze *et al*., 2015).

Estimation of titratable acidity: Sodium hydroxide (0.1 M, NaOH) solution was taken in the Burette. Ten ml of juice was diluted with 10 mL of distilled water and three drops of Phenolphthalein were added in the beaker. The same procedure was repeated for each citrus variety.

Estimation of Total soluble solids (Brix %): After evaluation and extraction of citrus juice from required fruit variety, few drops (an equal number of drops) of juice were dropped onto the refractometer prism plate. The reading was noted to one decimal place. Same procedure was repeated for all the varieties.

Determine Presence of Simple Sugars: In a test tube 10 drops of Benedict's solution and 10 ml of citrus juice (of required variety) were added. The test tubes were heated in hot water bath and observations were noted down in the form of color change. Concordant readings were taken for each variety.

Antioxidant Activity by DPPH Assay: one ml of all the dilutions i.e. 1 mL, 0.5 mL, 0.25 mL and 0.125 mL of all juices were taken in separate glass vials and two ml of freshly prepared DPPH was poured in each vial. Solution (0.1 mM) of DPPH was prepared in methanol and 1 ml of this solution was added to three ml of citrus juices (50-250 µg/mL) in methanol. After 30 minutes absorbance was measured at 517 nm, by using spectrophotometer. The percent DPPH antioxidant activity (by scavenging activity) was calculated by using following equation:

$$\text{Antioxidant activity (\%)} = [(A_C - A_S / A_C)] \times 100$$

A_C = Absorbance $_{Control}$

A_S = Absorbance $_{sample}$

Results & Discussion

In presented study the concentration of ascorbic acid was estimated in mausami, mandarin, grapefruit, lemon and nestle juice was 0.6 g, 0.5 g, 0.9 g, 0.7 g and 0.3 g in 20 mL of fruit extract respectively (Table 1) which is in line with the work of Nishanta *et al*. (2015) they estimated average concentration of ascorbic acid volumetrically in lemon, bitter orange, grapefruit and sweet orange in fruit extract.

In the presented work percentage acidity varied from 0.4 – 0.7 %. Total soluble solids (Brix %) in citrus fruits ranged between 7-10 % (Table 1) was determined by hand refractometer. The Brix % of mausami, mandarin, grapefruit, lemon and nestle juice was 9 %, 10 %, 7 %, 7 % and 10 % respectively (Table1 and Figure 1). The highest percentage was in tetra pack and grapefruit extracts. The antioxidant activity percentage was highest in grapefruit as compared to other citrus varieties. The absorbance values of citrus varieties such as kinnow, grapefruit, lemon and tetra pack juice were 46 %, 81 %, 60 % and 34 % respectively. Tetra pack juice had lowest absorbance percentage which was in line with the findings of Al-Juhaimi & Ghafoor (2013) they estimated the titratable acidity of citrus fruits i.e. lemon, mandarin and Orlando had titratable acidity, also evaluated the total soluble solids by digital refractometer. The estimated antioxidant activity of citrus fruits was estimated by DPPH radical scavenging activity.

Kumar *et al*. (2013) determined the reducing sugars with help of spectrophotometry. While in presented work, the reducing sugar in citrus sample varied from 0.1-2 % by using benedict reagent (Fig. 2). While in the presented work the citrus varieties mandarin and grapefruit had 0.1-0.5 % reducing sugar, mausami contained more than 2 % reducing sugar (Table 1).

Conclusion

The biochemical comparison of citrus fruit extracts and tetra pack juice elucidated that grapefruit juice was comparatively healthy among all citrus fruits due to it's the highest vitamin C content, percentage acidity and antioxidant activity while tetra pack juice was not healthy due the lowest vitamin C content and antioxidant activity.

Table 1: Comparison of different citrus fruit extracts and tetra pack juice.

Sr. No	Citrus Varieties	Acidity content %	Vitamin C content g/20 mL of juice	Brix %
1	Musami	0.4	0.6	9
2	Kinnow	0.5	0.5	10
3	Lemon	0.4	0.7	7
4	Grapefruit	0.7	0.9	7
5	Tetra Pack	0.5	0.3	10

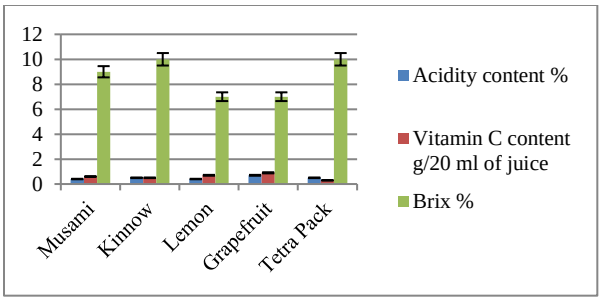

Fig. 1: Comparison of percentage acidity, vitamin C contents and brix percentage of citrus varieties with tetra pack juice.

Table 2: Square mean of four biochemical analysis of citrus fruits

SOV	df	Vitamin C	Reducing Sugar	Acidity %	Brix %
Var.	4	0.04067	2.781***	0.01767	6.44267***
Error	10	0.01867	0.046***	0.02667	0.10067***
Total	14				

***Significance P<0.001, **significance P<0.01, significance P>0.05

Fig. 2: Positive Benedict's test with citrus juices showing presence of reducing sugar in fresh juices except in tetra pack juice.

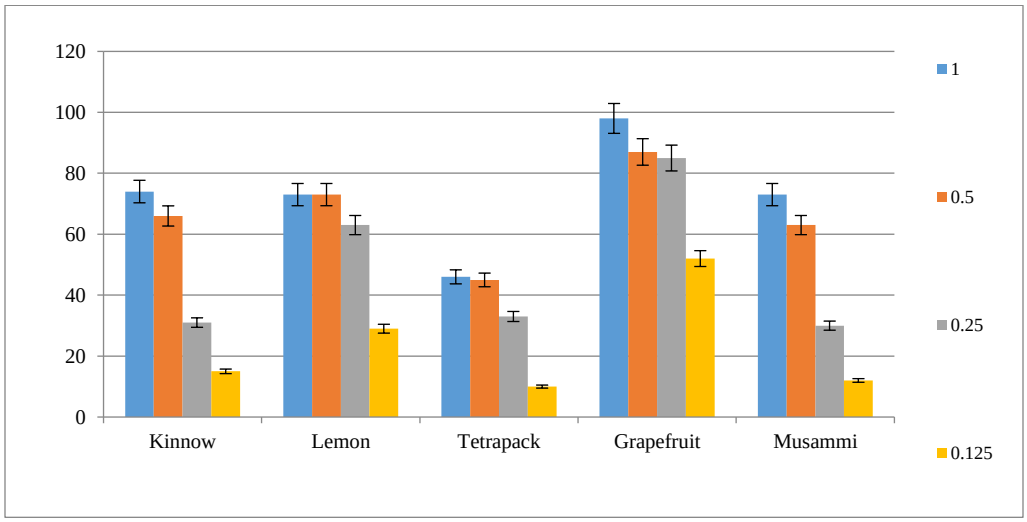

Fig. 3: Percentage inhibition of citrus varieties showing maximum antioxidant activity in grapefruit juice and lowest percent inhibition in tetra pack juice

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