Retention of organic acids in citrus juices during storage

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Abstract— Citrus is most important tree fruit crop grown in world and Pakistan is one of the leading citrus producing countries. Pakistani citrus cultivars particularly Kinnow (Mandarin) is famous throughout the world for its taste with increasing demand in international market. Organic acids play pivotal role in flavor and consumer acceptance. Organic acid are considered as mark of authenticity and helpful in detecting adulteration in citrus juices. Proper ratio of these organic acids accentuates the organoleptic attributes and accelerates the shelf stability of juices. Concentration of organic acids was determined through HPLC and their retention during storage was evaluated. Ascorbic acid, citric acid, malic acid and tartaric acid content ranged from 38.49 mg/100mL (red blood) to 51.43 mg/100mL (Kinnow), 916.52 mg/100ml (Kinnow) to 1321.60 mg/100mL (mussambi), 9.87 mg/100ml (mussambi) to 530.66 mg/100mL (Kinnow), 0.366mg/100mL (mussambi) to 0.842mg/100mL (Kinnow) respectively in different citrus species. Storage stability studies revealed that ascorbic acid, citric acid, malic acid and tartaric acid content varied between 38.49 mg/100mL (end of storage in mussambi) to 51.43 mg/100mL (at day 0 in Kinnow), 907.63 mg/100mL (30th day in Kinnow) to 1323.25 mg/100mL at initiation of storage (mussambi), 9.67 mg/100mL (at day 0 in mussambi) to 545g/100mL at end of experiment (Kinnow) and 0.318 mg/100mL at start of storage in mussambi to 0.843 g/100mL at the end of storage time (Kinnow), respectively for different citrus cultivars.

Index Terms— ascorbic acid, citrus, HPLC, juice authenticity, Kinnow, sensory, shelf stability

1 INTRODUCTION

Citrus belong to the family rutaceae and number 1 fruit with respect to area and production in Pakistan [1] (PHDEB 2005) contributing 2% to the overall world production. Pakistan ranked 9th for production of Tangerines, Mandarins and 11th for oranges among countries of the world [2] (FAO Stat, 2008). During year 2009-10, Pakistan produced 2,203 thousand tones citrus from an area of 160 thousand hectares [3] (GOP, 2010). Hot ambient temperature of tropical climates limits its shelf life [4] (Supraditareporn and Pinthong, 2007). Fruits are mostly used as fresh or processed for production of diversified products like juice.

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Juice is popular way to consume fruit and preferred by people of all age groups [5] (Nour et al., 2010). It is extracted from ripened fresh fruits during glut production and stored under controlled conditions [6] (Zvaigzne et al., 2009). Citrus juices are more convenient for customers due to their excellent food value and peptizing flavor, availability round the year and increased shelf life [7] (Nitu et al., 2010). It is most popular and extensively used all over the world [6] (Zvaigzne et al., 2009). Juice quality depends on the raw material, processing conditions, storage conditions and packaging materials [8] (Tariq et al., 2001). Citrus juices are good source of organic acids and supply phytonutrients, vitamins and minerals [6] (Zvaigzne et al., 2009). It is refreshing [9] (Roger, 2002) and contain several health promoting, favorable functional and valuable nutritional properties [10] (Okwu and Emenike, 2006). Inclusion of citrus juices in diet pattern is helpful in prevention of coronary diseases and chronic asthma [11], [12] (Abd-Ghafar et al., 2010 and Dugo and Giacomo, 2002).

Organic acids are second most abundant class of soluble solids in citrus juices and are helpful for prediction of maturity indices [5] (Nour et al., 2010) and their estimation is valuable for quality evaluation of citrus juices [13] (Hasib et al., 2002). Organic acids along with sugars affect the organoleptic quality characteristics of both raw and processed fruits [5] (Nour et

al., 2010). Some organic acids may be used as indicator of fruit ripeness [14] (Palmer and List, 1973), bacterial activity and adultration [15], [16] (Evans et al., 1983; Blanco et al., 1996). Ascorbic acid is the most valuable nutrient in citrus juices which is essential for the synthesis of collagen the most abundant protein in mammals [6] (Zvaigzne et al., 2009). Organic acids are involved in human growth, maturation and senescence [17] (Al-Farsi et al. 2005). The organic acids influence sensorial characteristics like flavor, color and aroma and are liable for various distinctive fruity tastes [18] (Magaia et al., 2013). These enhance shelf life, stability and microbial safety [13], [19], [5] (Hasib et al. 2002; Loredana et al. 2006; Nour et al. 2010). Citric and malic acids are main organic acids of citrus fruits [20] (Karadeniz, 2004) while tartaric, benzoic, oxalic and succinic acids are found in trace amounts [21] (Kale and Adsule, 1995). Organic acids are less susceptible to changing process and storage conditions. Qualitative and quantitative measurements are carried out for identification and quantification of individual acids in juice. Soluble solids of citrus fruits are meinly composed of organic acids and sugars [21] (Kale and Adsule, 1995) which are utilized as major index of maturity and main analytical measure of flavor quality [22] (Fellers, 1991).

Organic acid pattern is fruit specific and the concentration and ratios of these acids is helpful for quantification of the percentage juice and estimation of misbranding or adulteration in juices [5] (Nour et al., 2010). Authenticity of juices is verified from chromatographic evaluation of organic acids [23] (Camara et al., 1994) and individual fruit content in blended fruit juice beverages is estimated from level of acids [24] (Johnson et al., 1996). The composition, nature and concentration of organic acids in fruits are of particular interest due to their impact on the sensorial properties of citrus fruits and juices [5] (Nour et al., 2010).

In industry juice is stored at refrigeration temperature and by addition of preservatives. Juice characteristics like content of organic acids, sugars and organoleptic properties have been previously investigated. However, storage stability of organic acids in citrus juices has not yet investigated. Therefore, the present study was planned to determine the nature and amount of organic acids present in different citrus juices and to investigate the effect of storage on the retention of organic acids in absence of chemical preservatives.

2 MATERIALS AND METHODS

2.1 RAW MATERIAL: Different citrus species (Kinnow, Grapefruit, Blood Red and Musambi) were taken from local market. All the samples were stored at 5°C and were processed into fruit juice within a week. Fruits were washed in a tap water and then were peeled and divided into halves.

2.2 FRUIT PROCESSING: Fruit juice was extracted using a lab scale citrus juice extractor (Moulinex T574, France) by applying method described by Karadeniz [20] (2004). Then samples were cooled with tap water and were stored at -20°C until analysis. Different citrus varieties were evaluated at 0, 10, 20, and 30 days of storage for organic acid concentration.

2.3 SAMPLE PREPARATION: Samples were prepared following method developed by Karadeniz [20] (2004) with some modifications. Seven ml of juice was added to 40 ml of buffer-

acetonitrile mobile phase (0.5% (wt/vol) $(NH_4)_2HPO_4$ (0.038 M) -0.4% (vol/vol) acetonitrile (0.049 M), at pH 2.24 with H_3PO_4), extracted for 1 hour in orbital shaker (model 75, Burrell Scientific, Pottsburgh, PA) and centrifuged at 6000 x g for 5 minutes. The supernatant was collected and filtred once through whatman # 1 filter paper and twice through a 0.45-µm millipore membrane filter (Satorious SM 11606, Goettingen, Germany) and then 20µL was injected into HPLC . Analyses were performed in triplicate on all samples.

2.4 CHEMICALS AND STANDARDS: Among the chemicals used organic acids (Citric acid, malic acid, tartaric acid and ascorbic acid) were HPLC grade while Acetonitrile, potassium dihydrogen orthophosphate and phosphoric acid analytical grade, purchased from local suppliers of Merck and Sigma-Aldrich. The water used was ultrapure, Basic TWF. A standard stock solution was prepared by combining acids in follow-

ing portions (1000 mg/l citric acid, 2000 mg/l malic acid, ascorbic acid 700 mg/l and tartaric acid 400 mg/l). The stock solution and the corresponding dilutions was made in ultrapure water and stored in dark places between the experiments, at refrigeration temperature.

2.5 ORGANIC ACIDS DETERMINATION: Organic acids (ascorbic acid, citric acid, malic acid and tartaric acid) were determined by high performance liquid chromatography (HPLC) [20] (Karadeniz, 2004). Analysis was made by HPLC with UV detector (Perkin Elmer-series 200) at 214 nm using RP-18 column (120 x 4.6 mm). The operating conditions were: mobile phase, aqueous 0.5% (wt/vol) $(NH4)_2HPO4$ (0.038 M) - 0.2% (vol/vol) acetonitrile (0.049 M), then both solution were added 50, 50 % of each to make the final mobile phase, adjusted to pH 2.24 with H_3PO_4; flow rate 0.3 ml/min; ambient column temperature. The mobile phase was prepared by dissolving analytical grade $(NH_4)_2HPO_4$ in distilled water, HPLC-grade acetonitrile and H_3PO_4. HPLC grade reagents were used as standards (Sigma Chemical Co., St. Louis, MO). Solvents were degassed under vacuum filter through a 0.45-µm membrane. 20µl of sample was injected into HPLC for the analysis. First standards were run to observe the retention time of peak of specific acid. Four standards were run and separate peaks were taken then a combined peak of all the standards was taken. Then the samples were run and the peaks of samples were overlapped to see the desired acid in the sample.

2.6 STATISTICAL ANALYSIS: The data obtained was subjected to statistical analysis by using Completely Randomized Design (three factor factorial) and comparison of means was done by Duncan's Multiple Range Test [25] (Steel et al., 1997).

3 RESULTS AND DISCUSSION

Citrus juices are good source of certain volatile and non volatile organic acids. But most important non-volatile organic acids regarding the quantity are citric acid, ascorbic acid, malic acid and tartaric acid [26] (Farnworth et al., 2001). Among these acids ascorbic acid is most important due to its nutritional value for human health. An organic aicds profile provides valuable information regarding the authenticity of citric juice contents of drinks since they have lower susceptibility to change during processing and storage than other components of fruit [23] (Camara et al., 1994). Accurate knowledge of organic acids levels and ratio might be useful for determining the percentage juice content in juice products and also for detecting misbranding and adultration [27] (Coppola and Starr, 1986). Since each fruit has a unique pattern of organic acids [28] (Wrostald, 1981).

The identification of these organic acids was accomplished by comparing their retion time with those of authentic known standards of these acids. Standard chromatograms were prepared for each authentic acid. Four important acids i.e. citric, ascorbic, malic and tartaric were evaluated and identified.

Representative chromatogram of standard organic acid is given in Fig.1. The peak time 7.78, 8.54, 9.56 and 10.71 min represent tartaric acids, ascorbic acid, citric acid and malic acid respectively.

3.1 ASCORBIC ACID: Ascorbic acid is an important constituent of our nutrition because of its antioxidant capacity. During storage the loss of ascorbic acid from products is a critical factor for the shelf life of food product as citrus juices. The degradation of ascorbic acid proceeds both aerobic and

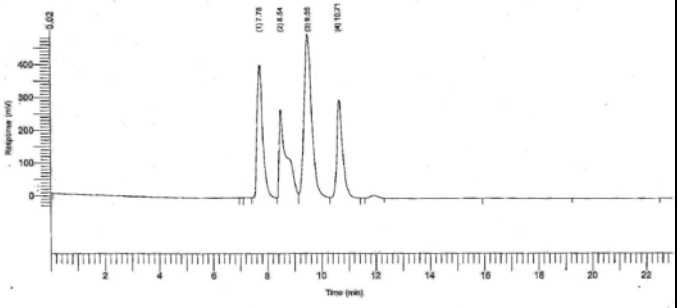

Fig.1. Chromatogram of (1)Tartaric acid,(2) Ascorbic acid, (3) Malic acid and (4) Citric acid analyzed by RP-HPLC Column C18 RP-ODS (25cm long, × 4.6mm i.d) in isocratic programme ; UV 214nm, mobile phase Acetonitrile: Ammonium Dihydrogen Phosphate (50:50), flow rate 0.3ml/min column temperature 30°C

anaerobic pathways and depends upon many factors such as oxygen, heat, light, storage temperature and storage time. Oxidation of ascorbic acid occurs mainly during the processing of fruit product, whereas, anaerobic degradation of ascorbic acid mainly appears during storage.

The ascorbic acid of the citrus juices was significantly influenced by storage period and the effect of treatment on ascorbic acid was also found to be highly significant. The first order interaction between storage time and treatment showed non significant influence on ascorbic acid. The significantly higher content of ascorbic acid was observed at the start of experiment (0 days) in all citrus varieties, kinnow was leading with mean value 51.43 mg/100mL while lower content of ascorbic acid was observed at the end of storage period (30^{th} day) in all cultivars; mussambi being lowest (38.49 mg/100mL) (Table 1). Results of the present study are well supported by previous findings of Nour et al. [5] (2010) and Ashurst [28] (1999) who found that the ascorbic acid content in range of 21.5 to 71.8 mg/100 ml and 26 to 84 mg 100-1 mL, respectively. Ascorbic acid content revealed decreasing trend in all the citrus varieties during storage. Results of current study are in accordance with previous findings by [29], [30], [4] Hoare et al. (1993), Johnston and Bowling (2002) and Supraditareporn and Pinthong (2007) who found that the ascorbic acid showed significant decrease in orange juices during a storage period even in unopened containers.

Significantly highest ascorbic acid content was noted in kinnow with mean value 51.43 mg/100ml while lower in blood red (31.74 mg/100ml). Similarly comparison of means of storage showed that there is maximum degradation of ascorbic acid at storage period of 30 days followed by 20 days with 38.42, 41.23 mg/100ml mean value of ascorbic acid (Table 1). The concentration of ascorbic acid reduced during storage of the juices [31], [32] (Lee and Coates, 1999; Johnston and Bowling, 2002). Significant decrease in ascorbic acid was observed in 59.4mg/100grams to 47.89mg/100grams T_0 (control), 52.38mg/100grams to 50.40mg/100grams in grapefruit and 59.40 mg/100 grams to 49.40 mg/100grams in kinnow. The ascorbic acid decreased significantly with increase in storage period ($P<0.05$) and maximum loss in ascorbic acid 46.70mg/100grams found after 45 days intervals [33] (Hussain et al. 2004).

The highest ascorbic acid content (51.43 mg/100ml) was estimated in kinnow at start of storage while lower in red blood after 30 days of storage period with mean value 31.47 mg/100ml (Table 1). The ascorbic acid content decreased in all varieties during 30 days of storage period. Minimum loss of ascorbic acid was noted in red blood which is 12.5 % while maximum in kinnow (25%) at the end of storage period of 30 days. Kabasakalis et al. [34] (2000) studied the ascorbic acid content of commercial fruit juices and its rate of loss upon storage and found loss of ascorbic acid was 29-41 % at room temperature for 4 months. However the commercial fruit juices when stored in open container in refrigerator the ascorbic acid loss was 60-67 % in 31 days storage. Sattar et al. [35] (1889) studied the effect of packaging materials and fluorescent light on HTST- pasteurized orange drink and observed that the ascorbic acid losses amounted 60.6%, 54.6%, 51.0% and 45.5% in clear glass, green glass, tetrapak and amber glass, respectively against 42.4% in the unexposed control during 32 days storage. The loss of ascorbic acid also depends upon the packaging material and storage conditions. Many factors affect the loss of ascorbic acid in processed orange juice including length of storage, temperature of storage and nature (oxygen barrier) of storage container [36], [37], [38] (Kanner et al. 1982, Marcy et al. 1989, Sadler et al. 1992).

Table: 1. Effect of storage on the ascorbic acid of citrus juices

Treatments	Storage Days				
	0	10	20	30	Mean
Kinnow	51.43	49.44	47.59	43.18	47.91
Grapefruit	46.39	45.54	43.61	40.27	43.95
Blood Red	36.38	35.44	33.44	31.74	34.25
Musambi	43.45	41.84	40.45	38.49	41.06
Mean	44.41	43.07	41.27	38.42	

3.2 CITRIC ACID: Citric acid is also an important organic acid regarding our diet and health. It works as preservative in fresh fruit juices and other food products because it works as antimicrobial agent. It is present abundantly in citrus juices as compared to other fruit products.

The effect of both storage period and treatments was found to be highly significant on citric acid content of the citrus juices. The first order interaction between storage time and treatment showed non significant influence on citric acid. Significantly higher citric acid content was noted in musambi with mean value of 1321.60 mg/100mL whereas lowest in kinnow (916.52 mg/100ml) (Table 2). Results of this study are line with previous findings reported by Nour et al. [5] (2010) that citric acid content ranged from 688.7 to 7393.6 mg/100ml among ten different citrus cultivars. Citric acid was most abundant organic acid in all citruc juices ranging from 6.05 to 60.32g/L except tangerine [20] (Karadeniz, 2004).

Significantly higher citric acid (1323.25 mg/100mL) was observed in mussambi at initiation of storage while the lower in kinnow after 30 days of storage period with mean value of 907.63 mg/100mL (Table 2). Similarly comparison of means of storage period showed maximum degradation of citric acid at end of storage period (30 days) with mean value of 1157.90 mg/100mL (Table 2). The data depicted slight decrease in amounts citric acid in all citrus juices during 30 days of storage period. The minimum loss in citric acid content (12.5 %) was recorded in red blood while it was maximum (25%) in kinnow at the end of storage period of 30 days. Over all, it is clear that the citric acid contents in citrus juices decreased slightly during storage period of 30 days.

Elez-Martinez et al. [39] (2006) reported that the citric acid contents in thermally treated orange juice stored at 4°C for 56 days ranges from 700 mg/ 100 ml to 710 mg/ 100 ml. It indicates that during storage of orange juice there is no significant change occurs in citric acid contents. The findings are close agreed with the values as reported by Farnworth et al. [26] (2001) who investigate that there is no decrease or increase occur in thermally processed orange juice stored at 4°C for two months to 9 months.

3.3 MALIC ACID: Malic acid is found in minute quantity citrus juices. It plays an important against cardiovascular dieses. It also has a little antioxidant properties. The statistical analysis Table 3 showed the influence of that storage period and treatments was highly significant while first order interaction between storage time and treatment. Among the different citrus varieties malic acid was significantly higher in kinnow with mean values of 530.66 mg/100mL and the lowest in mussambi with mean value of 9.87 mg/100ml.

Results of the current study are in close agreement with previous investigation by Nour et al. [5] (2010). He observed that malic acid content varied between 8.90 to 518.3mg/100ml in ten different citrus varieties evaluated. Maleic acid was second most abondunt acid in all citrus juices with value 1.27-12.15g/L [20] (Karadeniz, 2004).

The highest content of malic acid was observed at the end of experiment (30 days) in all citrus varieties with highest mean

Table: 2. Effect of storage on the citric acid of citrus juices

Treatments	Storage Days				
	0	10	20	30	Mean
Kinnow	925.92	919.21	913.32	907.63	916.52
Grapefruit	1312.11	1325.16	1318.21	1311.60	1316.77
Blood Red	1111.09	1110.37	1106.02	1098.38	1106.47
Musambi	1323.25	1329.03	1320.18	1313.95	1321.60
Mean	1168.10	1170.94	1164.44	1157.90	

Table: 3. Effect of storage on the malic acid of citrus juices

Treatments	Storage Days				
	0	10	20	30	Mean
Kinnow	519.62	525.00	533.00	545.00	530.66
Grapefruit	171.02	172.50	175.13	175.92	173.65
Blood Red	481.09	485.52	491.62	498.55	489.20
Musambi	9.67	9.76	9.86	10.18	9.87
Mean	295.35	298.20	302.40	307.41	

value (545g/100mL) in kinnow while lowest at the start of storage period (0 days) in all varieties with mean value (9.67 mg/100mL) in mussambi (Table 3). Increasing trend was observed in all the treatments along with storage time. Similarly comparison of means of storage showed that there is maximum increase of malic acid at storage period of 30 days with 307.41 mg/100ml mean value of malic acid (Table 3). The minimum increase of malic acid was in red blood which is 12.5 % while the maximum increase of malic acid was recorded in kinnow which is 25% at the end of storage period. The results of the present studies in close agreement with findings of Farnworth et al. [26] (2001) that there is an increase in malic acid contents in thermally processed orange from 917 to 992 mg/100 ml at 4°C for two months of storage.

3.4 TARTARIC ACID: The tartaric acid of the citrus juices was highly significantly influenced by storage period while the effect of treatment on tartaric acid was found to be non significant (Table 4). The first order interaction between storage time and treatment showed insignificant influence on tartaric acid. The significantly highest content of tartaric acid was observed at the end of storage time (30 days) in all citrus cultivars with highest in kinnow (0.843 g/100mL). The significantly lowest tartaric acid was observed at the start of storage period (0 day) in all varieties with mean value in mussambi (0.318 mg/100mL) (Table 4).

Table: 4. Effect of storage on the tartaric acid of citrus juices

Treatments	Storage Days				
	0	10	20	30	Mean
Kinnow	0.83	0.85	0.85	0.84	0.84
Grapefruit	0.64	0.67	0.67	0.66	0.66
Blood Red	0.37	0.37	0.37	0.37	0.37
Musambi	0.33	0.38	0.38	0.38	0.37
Mean	0.54	0.57	0.57	0.57	

No significant increasing trend was found in all the treatments along with storage time of 30 days. Significantly higher tartaric acid content was present in kinnow with mean values of 0.842 mg/100mL. Similarly comparison of means of storage shows that the effect of storage on tartaric acid is significant. Maximum value of tartaric acid was observed at storage period of 10 days 0.570 mg/100ml mean value of tartaric acid (Table 4).

The interaction between storage time and treatment showed that the combined effect of both factors on tartaric acid contents is non significant. Significantly the higher tartaric acid contents was in kinnow 0.854 mg/100ml at 10 days of storage while the lower in mussambi at the initiation of storage period with mean value of and 0.318 mg/100ml (Table 36). The minimum increase of tartaric acid was in red blood which is 12.5 % while the maximum increase of tartaric acid was recorded in kinnow which is 25% at the end of storage period of 30 days. Findings of this study are similar to previous study conducted by Nour et al. [5] (2010) who noted that tartaric acid content ranged from 1.20 to 37.6mg/100ml in ten different citrus varieties.

4 CONCLUSIONS

Organic acids play vital role in the preservation of juices. Oxidation and loss of ascorbic acid is a key factor for the shelf life of citrus juices. Ascorbic acid and citric acid were more susceptible to degradation during storage as their concentration decreased in subsequent analysis while increase in the concentration of malic acid was noted throughout storage period. Tartaric acid content showed an abrupt increase at 10th day and slight decrease in subsequent evaluations.

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