The Journal of Animal & Plant Sciences, 24(1): 2014, Page: 211-215
ISSN: 1018-7081

CHARACTERIZATION OF ORGANIC ACIDS IN JUICES OF SOME PAKISTANI CITRUS SPECIES AND THEIR RETENTION DURING REFRIGERATED STORAGE

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ABSTRACT

Organic acids play pivotal role in flavor and consumer acceptance of fruit beverages. Concentration of some organic acids in citrus juices was determined through HPLC and their retention during refrigerated storage was also evaluated. The concentration of organic acids were in the following pattern Citric Acid> Malic Acid> Ascorbic Acid> Tartaric Acid. Citric acid, malic acid, ascorbic acid and tartaric acid content ranged from 925.92 mg/100 mL (Kinnow) to 1323.25 mg/100 mL (Musambi), 9.67 mg/100 mL (Musambi) to 519.62 mg/100 mL (Kinnow), 36.38 mg/100 mL (red blood) to 51.43 mg/100 mL (Kinnow), 0.318 mg/100 mL (Musambi) to 0.825 mg/100 mL (Kinnow), respectively in different citrus species. Storage studies revealed that ascorbic acid losses were 11% to 15% and citric acid contents were decreased from 0.7% to 1.9% during refrigerated storage while malic acid contents increased 1% to 4% and tartaric acid content increased 2% to 4% thoroughout the storage period.

Key words: Characterization, Organic acids, Citrus juices, Refrigerated storage

INTRODUCTION

Citrus belongs to the family Rutaceae and ranks first with respect to total fruit production and area utilized in Pakistan. Pakistan ranked 13th for the production of oranges among countries of the world (FAOSTAT, 2011). During year 2009-10, Pakistan produced 2203 thousand tones citrus from an area of 160 thousand hectares (GOP 2010) and contributes 2% to the overall world citrus production. Hot ambient temperature of tropical climates limits its shelf life (Supraditareporn and Pinthong 2007). Fruits are mostly used as fresh or processed for production of diversified products like juices, marmalades, etc.

Citrus juices are good source of supplying phyto-nutrients, vitamins and minerals (Zvaigzne *et al*. 2009). It is refreshing (Roger 2002) and contains several health promoting, favorable functional and valuable nutritional properties (Okwu and Emenike 2006). Inclusion of citrus juices in diet pattern is also helpful in prevention of coronary diseases and chronic asthma (Abd-Ghafar *et al.* 2010; Dugo and Giacomo 2002). Citrus fruit extracts are also found to have antioxidant, anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities (Abeysinghe *et al.* 2007). These health benefits of citrus fruit have mainly been attributed to the presence of bioactive compounds, such as ferrulic acid, hydrocinnamic acid, cyanidin glucoside, hisperidine, vitamin C, carotenoid and naringin content (Abeysinghe *et al.* 2007; Xu *et al*. 2008).

Organic acids are second most abundant class of soluble solids in citrus juices and are helpful for prediction of maturity indices (Nour *et al.* 2010) and their estimation is also valuable for quality evaluation of citrus juices (Hasib *et al.* 2002). Organic acids along with sugars affect the organoleptic characteristics of both raw and processed fruits (Nour *et al.* 2010). Organic acid accumulation in the vacuole of cells of citrus fruits is a developmentally regulated process, the degree and timing of which varies greatly among species and varieties and is highly susceptible to agro-climatic conditions (Canel *et al*. 1995). Ascorbic acid is the most valuable nutrient in citrus juices and an essential component for the synthesis of collagen (Supraditareporn and Pinthong 2007). Citric, ascorbic and malic acids are main organic acids of citrus fruits while tartaric, benzoic, oxalic and succinic acids are found in trace amounts (Karadeniz 2004). Organic acid pattern is fruit specific and the concentration and ratios of these acids is also helpful for calculation of juice/pulp contents in the drink and estimation of misbranding or adulteration index in juices (Nour *et al.* 2010). Thus, 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 (Nour *et al.* 2010).

In citrus fruits the most important non-volatile organic acids regarding the quantity are citric acid, ascorbic acid, malic acid and tartaric acid (Farnworth *et al.* 2001). To the best of our knowledge the occurrence and distribution of organic acids in different Pakistani citrus species have not been investigated so far. Therefore, present study was undertaken to investigate the nature and amount of organic acids present in different citrus juices in order to determine the authenticity of juices. Furthermore, the effect of refrigerated storage on the retention of organic acids was also evaluated.

MATERIALS AND METHODS

Procurement of raw material and sample preparation: Different citrus species *Citrus reticulate*, *Citrus paraisi*, *Citrus sinensis* and *Citrus limetta* locally known as Kinnow, Grapefruit, Red blood and Musambi respectively were procured from Dhudhi Agri Farm, Sahiwal, Sargodha, Pakistan. All the fruits were grown on the same orchard and received similar agronomic practices. The samples were harvested early in the morning from the field and transported to National Institute of Food Science & Technology on the same day in ice coolers.

Fruit juice was extracted using a citrus hand juice extractor. After juice extraction, raw juice was heated at 96°C for one minute to inactivate enzymes. Following the heating process, the juice was rapidly cooled down to room temperature, filtered through 8-folded cheese cloth to eliminate particulates and then poured into 250 mL presterilised glass bottles. After bottling, all juice samples were again heated at 96°C for 2-3 min. Then samples were cooled with tap water and were stored at 4°C for 30 days. Different citrus varieties were evaluated at 0, 10, 20, and 30 days of refrigerated storage for organic acid determination.

Determination of organic acids: Organic acids (ascorbic acid, citric acid, malic acid and tartaric acid) were determined by high performance liquid chromatography (HPLC) by following the method of Akalin *et al*. (2002). Analysis was made by HPLC with UV detector (Perkin Elmer-series 200) at 214 nm using a reverse phase C-18 column (25 cm x 4.6 mm id).

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 as described by Steel *et al*., (1997) by using statistical software Minitab.

RESULTS AND DISCUSSION

Occurrence of organic acids in juice of citrus species: Results indicated (Table. 2) that among different organic acids determined in the current study, citric acid content was higher in all the citrus species. The concentration of organic acids present in citrus juice of different varieties were in the following order Citric Acid> Malic Acid> Ascorbic Acid> Tartaric Acid. The result of the study indicated that the citric acid content was higher in Musambi 1323.25 mg/100 ml juice and the lowest concentration of citric acid was found in Kinnow. The concentration of malic acid, ascorbic acid and tartaric acid were higher in Kinnow juice 519.62, 51.43 and 0.825 mg/100mL, respectively as compared to the other juices analysed. However, less quantity of malic acid and tartaric acid were found in all citrus species and lowest concentration of malic and tartaric acid was found in Musambi juice as 9.67 and 0.318 mg/100 mL, respectively.

The method used for extraction and quantification of organic acids from different citrus juices were validated and percent recovery of all organic acids was more than 90%. The results obtained during the course of study for different organic acids are described herein:

Karadeniz *et al.,* (2004) investigated organic acid distribution in nineteen authentic citrus juice species. The most abundant organic acid was citric acid, ranging from 605 to 6032 mg/100 mL and malic acid was recorded as the second most abundant organic acid with a range of 127-1215 mg/100 mL. Results of the current study are in close agreement with previous investigation by Nour *et al.* (2010) who studied the organic acids in natural and commercial citrus juices and showed that citric acid (688 to 7393 mg/100 mL) was the main organic acid in all citrus juices followed by malic acid (8.9 to 518.3 mg/100 mL) and ascorbic acid (21.5 to 71.8 mg/100 mL)) within citrus fruit species.

Significantly higher citric acid content was observed in Musambi (1321.60 mg/100 mL) whereas the lowest in kinnow (925.92 mg/100 mL). Significantly the higher citric acid (1323.25 mg/100 mL) was observed in Musambi at initiation of storage, while the lower in kinnow after 30 days of storage period (907.63 mg/100 mL) as depicted in Figure 1. Similarly, storage period showed maximum degradation of citric acid at the end of storage period (30 days) with 1157.90 mg/100 mL. The data depicted slight decrease in amount of 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. Overall, it is clear that the citric acid content in the juices of citrus species decreased slightly during storage period of 30 days.

The statistical analysis showed that the citric acid contents of citrus juices were found to be highly significantly affected by both storage period and treatments. The first order interaction between storage time and treatment showed non-significant influence on citric acid content (Table 1). Results of present studies are in line with previous findings reported by Nour *et al.* (2010) that citric acid content in ten different citrus cultivars ranged from 688.7 to 7393.6 mg/100 mL. Cancolan and Xu (2002) showed the same results in their storage study on orange juice and concluded that citric acid contents ranged from 600-1600 mg/100 mL which are close to the present study. Citrus species significantly affect the organic acid distribution of citrus fruit juices. Elez-Martinez *et al.* (2006) showed that the citric acid contents in thermally treated orange juice stored at 4°C for 56 days ranged from 700 mg/100 mL to 710 mg/100 mL. It indicates that during storage of orange juice no significant change occurs in citric acid contents. The findings are closely agreed with those reported by Farnworth *et al.* (2001) that no decrease or increase in citric acid occurs in thermally processed orange juices of different citrus cultivars stored at 4°C for 9 months.

Significantly highest ascorbic acid content was noted in Kinnow (51.43 mg/100 mL) while lower in Red Blood (36.38 mg/100 mL). The significantly higher ascorbic acid contents was observed at the start of experiment (0 days) in all citrus varieties, Kinnow was leading with 51.43mg/100mL while lower ascorbic acid content was found at the end of storage period (30 days) in all cultivars; Musambi being the lowest 38.49mg/100mL (Fig. 2). Similarly, storage showed that there is maximum degradation of ascorbic acid at storage period of 30 days followed by 20 days with 38.42 mg/100mL and 41.23mg/100mL, respectively.

The statistical analysis showed that the ascorbic acid contents of citrus juices were significantly affected by storage period and the effect of treatments (citrus species). However, first order interaction between storage time and treatment showed non-significant influence on ascorbic acid (Table 1). Results of the present study are supported by previous findings of Nour *et al.* (2010), they reported ascorbic acid content in the range of 21.5 to 71.8mg/100mL and 26 to 84mg/100mL in two different citrus species during storage. Citrus juices are rich sources of ascorbic acid, which is an important antioxidant. During storage the loss of ascorbic acid from food products is a critical factor for the shelf life of fruit juices. The degradation of ascorbic acid proceeds both aerobic and 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. Ascorbic acid content revealed decreasing trend in all the citrus varieties during storage. Results of current study are in accordance with previous findings by Johnston and Bowling (2002) and Supraditareporn and Pinthong (2007) who found that the ascorbic acid showed significant decrease in orange juices during storage period even in unopened containers.

Among the different citrus varieties, malic acid was significantly higher in kinnow (519.62 mg/100mL) and the lowest was observed in musambi (9.67 mg/100mL). Figure. 3 indicated that the highest content of malic acid was observed at the end of experiment (30 days) in all citrus varieties with highest mean value (545 mg/100mL) in kinnow while the lowest at start of storage period (0 days) in all varieties (9.67mg/100mL) in musambi. Increasing trend was observed in all the treatments along with storage time. Similarly, comparison of means during storage showed that there is maximum increase of malic acid at storage period of 30 days with 307.41mg/100mL of malic acid (Fig. 3). The statistical analysis (Table 1) showed that influence of storage period and treatments was highly significant while the interaction between storage time and treatment was non-significant. Results of the current study are in close agreement with previous investigation by Nour *et al.* (2010). They observed that malic acid content varied between 8.90 to 518.3mg/100mL in ten different citrus varieties evaluated. The results for malic acid showed a close relationship with the findings of Cancolan and Xu (2002) who showed that on average malic acid was 268.1 mg/100mL with a wide range from less than 112.4 to more than 367.4 mg/100mL. 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 are in close agreement with findings of Farnworth *et al.* (2001) that there is an increase in malic acid contents in thermally processed oranges from 917 to 992mg/100mL at 4°C for two months of storage.

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 (0.825 mg/100mL). The significantly lowest tartaric acid was observed at the start of storage period (0 days) in all varieties (0.318mg/100mL) in musambi (Fig. 4). Non-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 (0.842mg/100mL). Maximum value of tartaric acid was observed at storage period of 10 days 0.570mg/100mL mean value of tartaric acid.

The statistical analysis given in Table 1 showed that 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. The first order interaction between storage time and treatment showed non-significant influence on tartaric acid. 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 content was in kinnow 0.854mg/100mL at 10 days of storage while the lower in musambi at the initiation of storage period with 0.318mg/100mL (Fig. 4). 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*. (2010) that tartaric acid content ranged from 1.20 to 37.6 mg/100mL in ten different citrus varieties.

Table 1. Anova for concentration of organic acids in juices of various citrus species during storage

SOV	df	MS			
		Ascorbic acid	Citric acid	Malic acid	Tartaric acid
Storage (A)	3	398.135**	450908.09**	756549.14**	0.652^{NS}
Treatments (B)	3	80.671**	331.035**	331.035**	0.002^{NS}
A X B	9	1.676^{NS}	74.097^{NS}	74.097*	4.954^{NS}
Error	32	13.917	31.562	31.562	0.001
Total	47				

* = Significant (P<0.05); ** = Highly Significant (P<0.01); ^{NS} = Non-Significant (P>0.05)

Table 2. Occurrence of organic acids in juice of citrus species (mg/100ml)

Treatments	Citric acid	Ascorbic acid	Malic acid	Tartaric acid
Kinnow	925.92±37.03	51.43±2.06	519.62±20.78	0.825±0.03
Grapefruit	1312.01±52.48	46.39±1.85	171.02±8.55	0.639±0.04
Red blood	1111.09±55.55	36.38±2.18	481.09±14.43	0.372±0.02
Musambi	1323.25±79.39	43.45±1.73	9.67±0.58	0.318±0.01

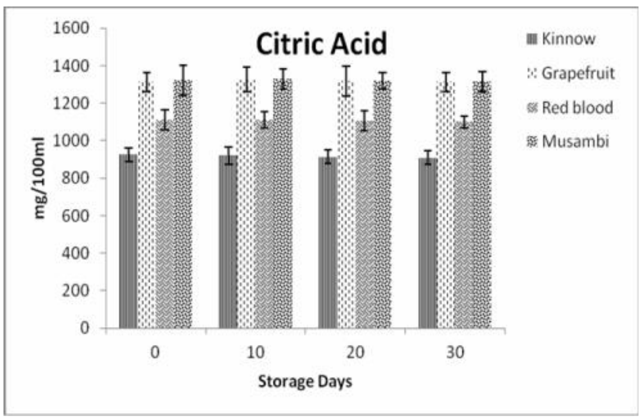

Figure 1. Effect of storage on the citric acid of citrus juices

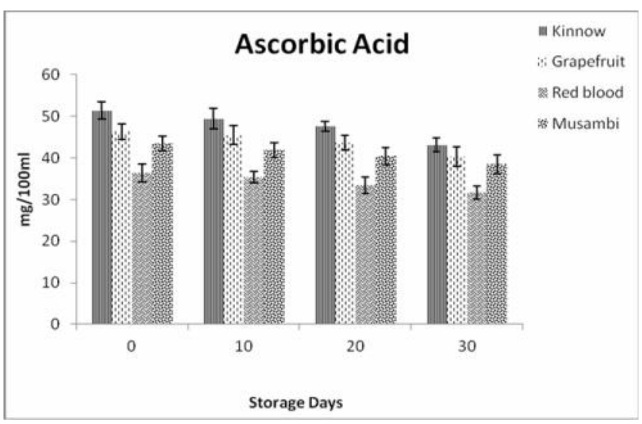

Figure 2. Effect of storage on the ascorbic acid of citrus juices

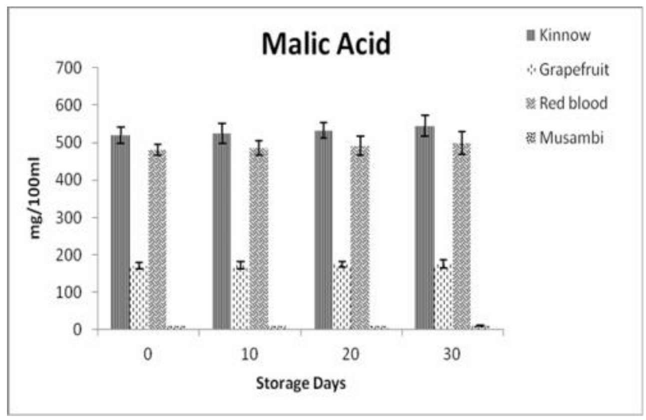

Figure 3. Effect of storage on the malic acid of citrus juices

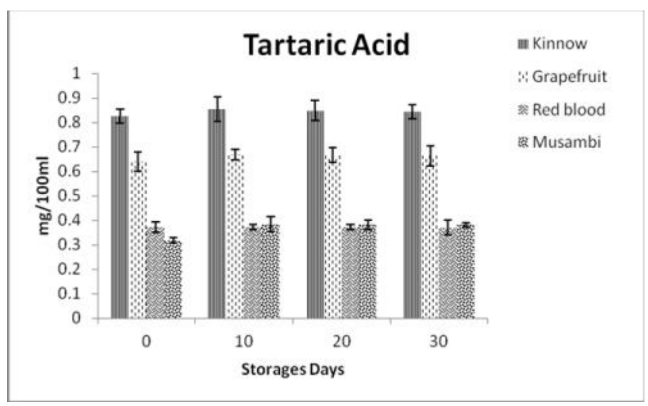

Figure 4. Effect of storage on the Tartaric acid of citrus juices

Conclusion: Organic acids play vital role in the preservation of juices. Oxidation and loss of ascorbic acid is a key factor determining 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 observed throughout storage period. Tartaric acid content slightly increased at 10^{th} day of storage and then decreased near to the original value at the end of storage period.

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