Research Article

Postharvest Physiological Disorders and Organoleptic Properties in Relation to Fungal Disease Incidence in Citrus

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Abstract | Citrus (*Citrus sinensis*) is a prominent fruit being produced and consumed in Pakistan. Its quality is largely influenced by postharvest handling and conditions that imparts huge economic and health implications when not been addressed properly. This study has been attempted to assess the quality of citrus in terms of postharvest physiological disorders and organoleptic properties in relation to the occurrence of pathogenic diseases. Fruit samples (injured and healthy) were taken from the three different local markets of Karachi. Some of the fungal species viz. *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Rhizopus stolonifer* and *Alternaria alternata* have been identified in citrus samples. However, major incidence was recorded for *Aspergillus niger* (41.6%) followed by *Fusarium oxysporum* (27.7%). Infected samples (5.9) had high pH as compared to the healthy samples (3.1). Total soluble solids were decreased from 14 to 8.5% due to fungal infection. Whilst, the infected samples had increased sugar to acidity ratio. The correlation coefficient (*r*) has also confirmed the influence of fungal disease on postharvest physiological disorders and organoleptic properties. The overall acceptability of citrus had strong relationships with pH (r = -0.95**), total soluble solids (r = 0.96**), Acidity (r = 0.93**) and sugar/acid ratio (r = -0.93**). Findings of the study would be suitable for addressing the incidence of identified pathogenic fungi. It will be useful to enhance quality and to extend shelf life of citrus for local as well as export oriented markets.

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1. Introduction

Citrus fruit is popular as a source of vitamin C, flavonoids and phenolic compounm ds. It is a leading fruit being produced in the Pakistan and shares about 30% of total fruits production in the country. Most of the citrus is being consumed locally and about 10% of total production is exported to other countries. Substantial losses (20 to 50%) have been recorded due to improper post-harvest handling and practices (Olsen *et al.*, 2000; Milind, 2008; Droby, 2006; Zhu, 2006). Mechanical damage likely to occur at the time of fruit handling (Sanches *et al.*, 2008) and resulted into unfavorable changes in fruit quality, taste, nutritional value, color and shelf life (Durigan *et al.*, 2005) and decrease in the vitamin C content (Lee and Kader, 2000). The deleterious cause of mechanical injury is decreased in acid content of fruits such as tomato, guava, mango and peach has been reported (Mattiuz and Durigan, 2001; Durigan

et al., 2005; Kasat *et al*., 2007). Improper storage and transportation are also known to provoke growth of microorganisms due to changes in physiological condition of fruits and vegetables (Singh and Sharma, 2007).

Quality of citrus like other fresh fruits can be assessed through color, firmness, total soluble solids, total acid and sugar-acid ratio characters etc. Low pH, higher moisture content and nutrient composition of fruits induces pathogenic fungi, which make them less palatable (Moss, 2002; Olsen *et al*., 2000; Milind, 2008; Singh and Sharma, 2007). Infection can be easily transferred from single to groups of adjacent fruits. (Jay, 2003). *Aspergillus spp.* Produces mycotoxin that is unsafe for humans and animals' life (Hejri *et al*, 2013). During packaging, *Penicillium species* enters into the tissue and deteriorates the fruit quality.

Annually, diseases from fungal pathogens causes severe losses to production of agricultural and horticultural crops every year (Parveen *et al*., 2016). Successful postharvest management is a potential solution which requires certain activities to be planned and performed at appropriate time. Initially, scientific based information is required on the incidence of diseases and their influence on fruit quality. This study has therefore been designed to identify the main fungal threats to the locally produced citrus and their quality indices being affected from pathogenic fungal attacks.

2. Materials and Methods

2.1 Collection of samples

The experiment was carried out to evaluate the incidence of postharvest physiological disorders, fungal contamination and changes in organoleptic characteristics of the citrus (*citrus sinensis*) quality. Thirty six samples of citrus fruit were collected from three different local markets namely new Sabzimandi (market 1), Saddar (market 2) and Liaquatabad (market 3) of Karachi. Considering physical appearance, sample representing each market contained six injured and six healthy fruits. The collected samples were transported to the lab in sterile polythene bag with proper packing and labelling.

2.2 Sample preparation

Samples were washed with 0.01% sodium hypochlorite (NaOCl) solution to avoid microbial contamination on surface and dried at room temperature.Samples were peeled off and seeds were separated from pulpy portion prior to blending.

2.3 Culture media preparation

Potato Dextrose Agar (PDA) is used as general culture media for isolation of fungi. A 39 g of commercial PDA powder was added to 1L of distilled water. Chloromphenicol (30mg/L) was added to prevent the bacterial growth. The mixture was dissolved under continuous stirring and boiling. Culture media was autoclaved for 15 min at 121°C.

2.4 Isolation of fruit spoilage fungi

Samples were cut into small pieces (2-3mm). The pieces were then transferred to sterilized and solidified media plates of potato dextrose agar (PDA). These plates were completely wrapped with parafilm and placed in incubator. The inoculated plates were incubated at 25-30°C for 5-7 days. The growth of fungal colonies was observed and recorded. The cultures were then subjected to purification according to the procedure described by (Parey *et al*., 2013).

2.5 Identification of isolated fungi

The fungal colonies were identified on the basis of macroscopic properties (colour, shape and appearance) and microscopic characteristics (like conidia shape, hyphae septations and other structures). The microscopic examination was undertaken under microscope at magnification of 10X and 40X (Tafinta *et al*., 2013; Samson and Varga, 2007).

2.6 Physiological quality analyses

Samples were examined for physiological parameters viz. total soluble solids, titratable acidity and pH. Digital refractometer (Hanna's, HI 96801) was used for total soluble solids. Titratable acidity was determined through titration method. The pH values were measured with bench top pH meter (HANNA, HI 3512, USA). The sugar acidity ratio was also calculated by dividing the Total Soluble Solids values with acidity values (AOAC, 2010).

2.7 Organoleptic evaluations

Organoleptic evaluation of samples was carried out by a trained panel of 12 judges by following the method recommended by Larmond (1977). Each sample was presented to judges in random order. Judges evaluated the samples by assigning scores ranges from 1-9, where 1 and 9 represent extremely "dislike" and extremely "like", respectively. On the basis of total organoleptic score, the samples were classified as "excellent" (score ≥ 8), "good" (score between 6-8), "fair" (score ≥5) and

"poor" (score ≤ 5).

2.8 Statistical analysis

Samples were analyzed in triplicate for each parameter. Mean and standard deviations were determined. Correlation coefficients (*r*) were evaluated between the test parameters to assess the dependency of parameters on each other. The statistical analyses were performed by using SPSS software (SPSS version 17, Inc., USA).

3. Results and Discussion

This study conducted to evaluate specific fungal incidence and physico-chemical changes in injured and healthy fruits. Different quality parameters including pH, total soluble solid (TSS), percent acidity, sugar-acid ratio and organoleptic properties were evaluated in relation to pathogenic incidence.

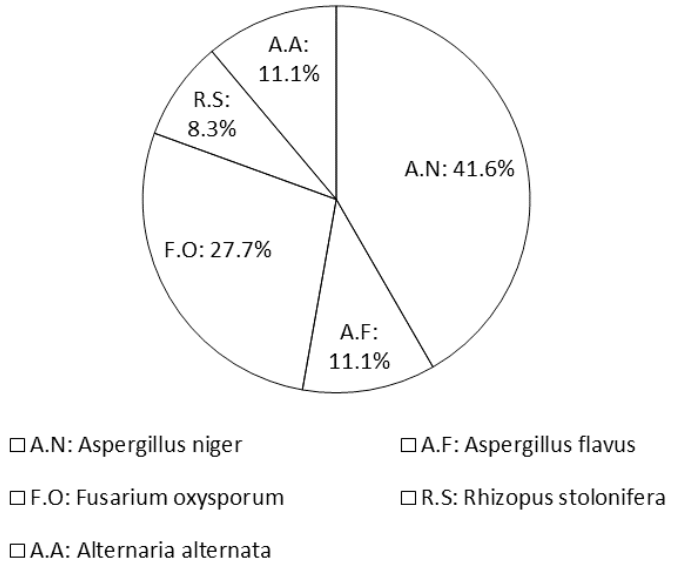

Figure 1: Percent incidence of fungal pathogens in citrus.

Figure 1 shows the percent incidence of fungal pathogens in citrus. Results show that five different species of fungi viz. *Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer, Aspergillus flavus and Alternaria alternata* were identified. The most prevalent species were found to be *Aspergillus niger* with the incidence of 41.6%. *Aspergillus spp.* are one of the most common species found on different kinds of fruits like apple and grapefruits, oranges and mango (Abdullah *et al.*, 2016). Long time transportation and high humidity supports the growth of *Aspergillus spp.* rot of fruits. Temperature plays an important role in the activities of fungi, high temperature enhances the spoilage of fruits (Fatima *et al.*, 2012). In the present study, the second most prevalent species was *Fusarium oxysporum* (27.7%). Whilst, the lowest incidence was observed for *Rhizopus stolonifer* (8.3%).

Figure 2 presents the detailed number of infected samples found in three selected markets. Three species viz. *Rhizopus stolonifer, Aspergillus flavus and Alternaria alternata* were rarely identified in one fruit market. The *Aspergillus spp.* is underlying mediator of post-harvest deterioration of fresh fruits in Pakistan. Sugary flavor and low pH level of acidic fruits favors the attack by *Rhizopus spp.* (Al-Hindi *et al.*, 2011; Gadgile and Chavan, 2010; Diedhiou *et al.*, 2007; Muhammad *et al.*, 2011).

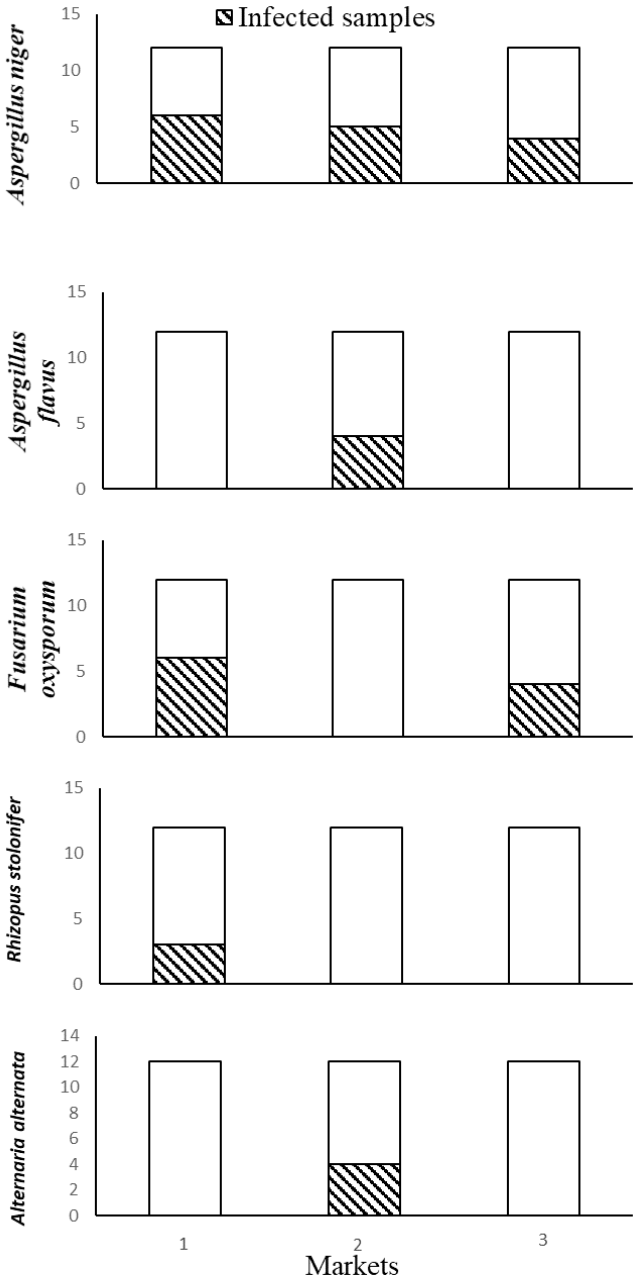

Figure 2: Number of market samples infected by different pathogens.

Figure 3 shows the physiological disorders of infected citrus and their comparison with the healthy citrus. A remarkable difference can be seen between infected and healthy citrus in terms of their pH, TSS, acidity and sugar to acid ratio. Total soluble solids of infected citrus (8.8%) were significantly lower than the healthy citrus (13.6%). Reduction of total soluble solids in injured fruits can be related to the use of these compounds as respiratory substrate since there is an increase in CO_2 production in relation to intact fruit (Mattiuz and Durigan, 2001). Also, sugary flavor in the citrus fruits attracts the pathogens for their growth and contamination. Higher temperature increases the metabolism rate and hydrolysis of starch that finally decreases the TSS (Thompson, 1996).

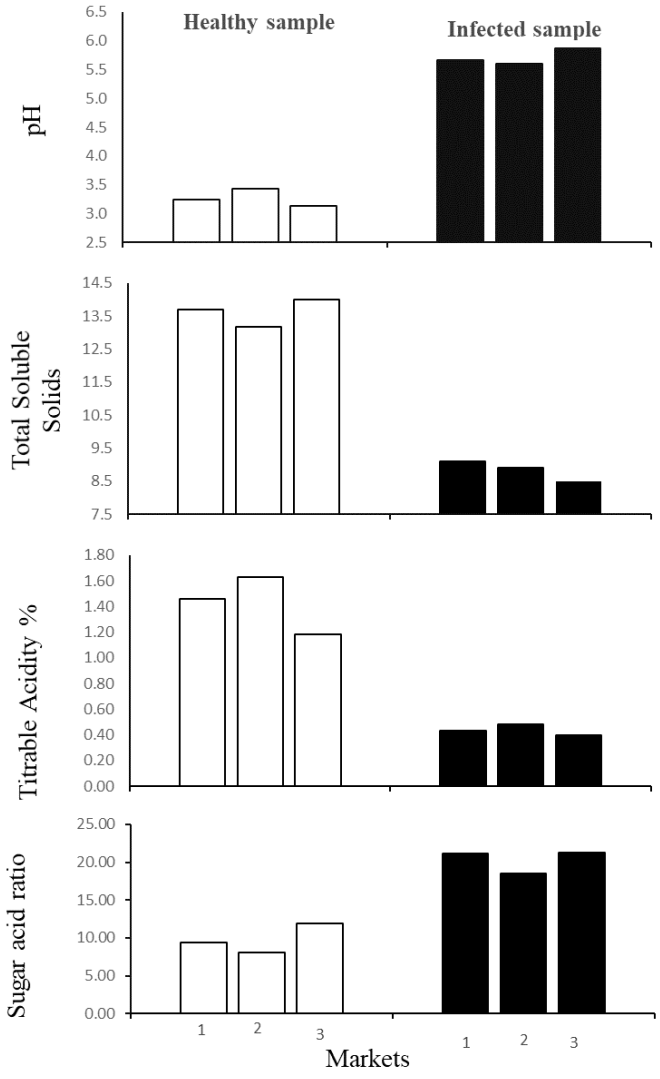

Figure 3: Effect of fungal infection on physiological parameters of citrus.

Titratable acidity is a key attribute of fruits quality that reveals either condition is favorable or adverse for fungal growth. In this study, it was found to be higher in healthy citrus as compared to infected samples (Figure 3). Besides increasing level of maturity, the infection and injury also leads to the reduction in acidity of fruit (Abbasi *et al*., 2009; Padayatty *et al*., 2003; Rickman *et al*., 2007). Alternately, the pH values were found lower in infected samples as compared to healthy samples. The differences were statistically significant ($p<0.05$).

Compositional changes also affected the organoleptic quality of fruit leading towards the change in acid and sugar ratio that directly influence the taste of fruit. We have also found strong relationships (r = 0.84 to 0.99) between physiological and sensory attributes of citrus (Table 1). Iincidence of fungal infection is an indication of fruit injury by physical or mechanical and over ripening of fruits that alter the physiological condition of fruit and ultimately change the sensory attributes of fruit.

Table 1: Relationship between physiological and organoleptic properties of healthy and infected citrus.

Quality parameters	Color	Texture	Taste	Overall acceptability
pH	-0.87	-0.94	-0.95	-0.95
TSS	0.87	0.95	0.99	0.96
Acidity	0.86	0.85	0.91	0.93
Sugar-acid ratio	-0.85	-0.84	-0.91	-0.93

Table 2: Sensory evaluations of infected and healthy citrus fruits.

Sample type		Sensory attributes			
		Color	Texture	Taste	Overall Acceptability
Infected fruit	1	5	5	5	5
	2	5	4	5	5
	3	5	4	5	4
	Mean	5 [a]	4 [a]	5 [a]	5 [a]
Healthy fruit	1	7	8	7	8
	2	8	7	8	8
	3	8	9	8	8
	Mean	8 [b]	8 [b]	8 [b]	8 [b]

Sensory scoring: ≥ 8: excellent score, 6-8: good score, ≥5: fair score and ≤ 5: poor score; Mean value with different letters represents statistically significant ($p<0.05$) difference.

Results of sensory evaluation of infected and healthy citrus samples are given in Table 2. All the sensory attributes including colour, texture, taste and overall acceptability were significantly influenced ($p<0.05$) by

fungal infection. Fungal infection changes the colour of fruit, brown to black infected lesions produced by *Alternaria spp*. Further, such fungal spoilage changes the aroma, taste and texture of fruit and become undesirable for human use (Gorny and kader, 1996). Cold weather dried the fruit pulp and brownish spots are appear on the outer surface of fruits. Yellow to brown leathery areas developed due to sunburn. Pesticide or herbicide injury causes necrotic spots on fruits that resemble with rot (Begeman and Wright, 2009). Improper cultural practice and Over nitrogen dose can produce thick peel of orange that influence on quality (Corinne Rhodes, 2014). Cracks produce on skin during handling and harvesting allows the fungus entry therefore, rough handling produce sunken spots on upper layer of fruit (Hui and valley, 2008).

Author's Contribution

All author of the manuscript contributed equally in overall planning, sample analysis, data interpretation and writing of research article.

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