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Original Research Article

Postharvest Treatment for Preserving Antioxidant Properties and Total Phenolic Content of Tomatoes and Litchis

Jyoti Singh, Brototi Roy*, Jaldhi, Shruti Mishra and Rashi Garg
Department of Zoology, Maitreyi College, Chanakyapuri, New Delhi-110021
*Correspondence: brototiroy@gmail.com

ABSTRACT

Fruits and vegetables are rich in antioxidants and polyphenols that help in prevention of many disorders like non-communicable and degenerative diseases. However, they are highly susceptible to bacterial and fungal spoilage. In light of this, the present study aims to quantify the loss of antioxidant properties of litchis and tomatoes stored in the refrigerator for 21 days and to explore the protective effect of UV-C and hot water immersion treatment (HWT) to prevent, or atleast reduce, the effect of storage. Interestingly, both the treatments were able to reduce the loss of antioxidant properties in stored tomatoes, whereas, UV-C and HWT completely abrogated the storage-induced loss of antioxidant properties in litchis.

Keywords: Antioxidant activities, Total phenolic content, Postharvest Treatment, UV-C treatment, Hot water immersion treatment

1. INTRODUCTION

Fruits and vegetables can be considered as a vital reservoir of nutrients including vitamins, minerals and antioxidants. The intake of fruits and vegetables is correlated with a decrease in the incidences of a plethora of diseases. India is the second-largest producer of vegetables and fruits next only to China, as reported by the Press Information Bureau of India in the year 2017 (Singh, 2017). Fruits and vegetables are highly susceptible and thus perishable due to bacterial and fungal infections. Not only is the spoilage an acute wastage of food, but it also represents a similar waste of human effort, farm inputs, livelihoods, investments and scarce resources such as water. 20-40% of the total production goes waste even before reaching the consumers (Shahi, 2009). Only 2% of fruit and vegetable production undergoes processing and majority of the product gets spoiled. In fact, according to recent estimates by the Food and Agricultural Organisation (FAO), approximately 1.3 billion tonnes of all food produced globally gets wasted (2019). It is thus necessary to make it available to the consumers all around the year in an edible form, without depletion of their antioxidant activities and total phenolic content that attribute to their high nutrition value. Advances in the biochemistry and molecular biology of the fruit ripening process has enabled the development of biotechnological strategies for the preservation of postharvest shelf life and quality of fruits, vegetables, and flowers. Several metabolic changes are initiated after the harvest of fruits and vegetables (El-Ramady *et al.,* 2015). Post-harvest methods or technologies can be useful tools to meet global or large scale local demand. These methods result in a slowdown of physiological processes of maturation and senescence, and reduce the risk of microbial growth (Mahajan et al., 2014). Postharvest technologies can include physical, thermal, chemical or gaseous treatment. Chemical treatment, however, proves detrimental to human health (James & Zikankuba, 2017; Damalas & Eleftherohorinos, 2011).

Exposure to ionizing radiation for a long duration can have harmful biological effects, but the same radiation (UV-B/C) can also protect if the exposure time is limited. UV-C radiations are non-ionizing radiations falling in the wavelength band of 200-280 nm. The beneficial doses have been reported to induce the phytoalexin production and accumulation, and activate genes of stress-related proteins (Maharaj, 2015). Another commonly used method for long-term storage is keeping fruits and vegetables at low temperatures. However, this method can cause freezing and chilling injury. The initial response to low temperature is considered to involve physical factors such as membrane alteration and protein/enzyme diffusion, but physiological changes like the dissolution of middle lamella polysaccharides can affect texture and taste, thus altering overall quality of the fruit/vegetable. Exposure to optimum temperature initially, followed by storage at 4 ^{o}C, also referred to as heat shock treatment, can provide a feasible alternative to cold storage of fruits and vegetables. The beneficial effect of HWT is thought to be due to heat inactivation of degrading enzymes and by destruction of insects and fungal pests (Mahajan et al., 2015). It involves washing fresh fruits and vegetables (FFV) in hot water (37-55 ^{o}C) for a duration ranging from 30 seconds to 2 hours depending on the cultivar, size, maturity and condition of FFV.

In the present study, an effort was made to quantify the morphological deterioration and estimate the loss of antioxidant activities and total phenolic content of a common fruit litchi and vegetable tomato stored in the refrigerator for different durations. Both tomatoes and litchis are common in the Indian subcontinent. In addition, tomatoes are known to be rich in lycopenes (a type of β-carotenes), polyphenols etc. (Agarwal and Rao, 2000) Similarly, a tropical fruit such as litchi has an abundance of low molecular weight polyphenols. The antioxidant property of tomatoes and litchis is attributed to the presence of these compounds. Hence, the present study attempts to assess whether the deterioration of morphological features could be used as an index for determining the loss in nutrients such as antioxidants and phenols. Importantly, the study explores the efficacy of simple, cost-effective, non-chemical postharvest techniques such as UV-C and HWT as a means of preserving the antioxidant properties of stored fruits and vegetables.

2. MATERIAL AND METHODS

2.1. Chemicals and Reagents

2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu reagent (FCR), Gallic acid, Ascorbic acid (AA), Sodium acetate, Glacial acetic acid, Ferric chloride and Sodium carbonate were purchased from Merck. All the chemicals used were of analytical grade.

Tomatoes and litchis were procured fresh from farms in Delhi and NCR. All fruits were washed with chlorinated water (0.1% Sodium hypochlorite solution, pH 7.0 for 3 min) and dried at room temperature. They were separated into 12 groups of five items each. Group 1-4 were untreated, 5-8 were UV-C irradiated and 9-12 underwent HWT (Figure 1). For UV-treatment, FFV were kept under UV light for 15 minutes to reach energy levels of 12.5 KJ per square meter (Andrade-Cuvi *et al.*, 2017). For heat shock treatment, FFV were kept in hot water at 40^{o}C for 10 minutes followed by immediate storing at 4^{o} C. They were then dried and all the groups, namely untreated, UV treated and heat shock treated, were kept in labeled containers, covered with a muslin cloth and refrigerated for different durations. An assessment of various parameters was made at regular intervals.

2.2. Deterioration index

The deterioration of tomatoes and litchis was determined by the method of Andrade-Cuvi *et al.* (2017) in which visual inspection using a four-point hedonic scale (1=no damage, 2=mild damage, 3=moderate damage, and 4=severe damage) was used. The main symptoms assessed were pitting, dehydration, loss of firmness, browning and decay. The deterioration index (DI) was calculated according to the following equation:

$$DI = \frac{\sum(\text{Damage level}) \times (\text{No. of trays per level})}{(\text{Total No. of trays evaluated})}$$

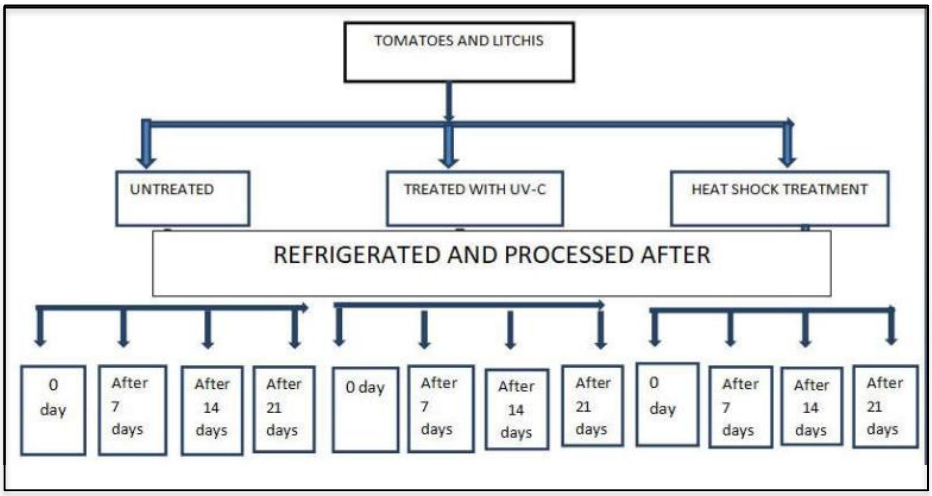

Figure 1: Schematic representation of the experimental design

2.3. Assessment of Total Phenolic Content and Antioxidant property

The important active substances in fresh fruits and vegetables are polyphenols, which have antioxidant, anti-mutagen, anti-inflammatory and antimicrobial abilities. The antioxidant activity of phenolic compounds is mainly due to their reducing properties which allows them to act as metal chelators that absorb and neutralize free radicals. TPC of the samples was quantified by using the method of Singleton and Rossi (1965) and Sasic *et al.* (2012) which depends on the reduction of Folin-Ciocalteu reagent by phenolic compounds under alkaline condition. Briefly, 0.2 ml of the diluted sample was taken in a test tube and mixed with 1:10 diluted FCR. The contents of the tubes were vortexed and allowed to stand for 10 min. Then, 0.8 ml of sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for 30 min. The absorbance of the resulting solution was measured at 700 nm. TPC was expressed as GAE (gallic acid equivalents) in mg/100 ml of the sample. The concentration of polyphenols was determined from the standard curve of gallic acid.

Ferric ion reducing power (FRAP) assay was performed to evaluate the antioxidant activity in tomatoes and litchis by using the previously standardized method of Roy *et al*. (2020). FRAP assay measures the ability of antioxidant compounds to reduce Fe(III) to Fe(II) under acidic conditions. In a test tube, 0.4 ml of the sample was mixed with 1.6 ml of working FRAP reagent. The reagent was prepared by a modified method of Benzie & Strain (1996). The stock solutions of acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in 40mM HCl) and 20 mM $FeCl_3$ were mixed in a ratio of 10:1:1. The tubes were further incubated at 37 °C for 30 min. The optical density of the coloured ferrous tripyridyltriazine complex in the solution was measured at 590 nm. Ascorbic Acid (AA) was used as standard. The results were expressed as mg/100 ml of AA.

2.4. Statistical analysis

All the samples were run in triplicate. Standard deviation and standard error of mean were calculated and expressed in respective graphs. Analysis of variance was done using GraphPad Prism Version 5. Also, p-value < 0.0001 was considered significant.

3. RESULTS AND DISCUSSION

3.1. Effect of storage on morphological characteristics, antioxidant activities and TPC of tomatoes and litchis

21 days of refrigeration had adverse effects on the morphology of both, untreated tomatoes and litchis. The litchis showed browning, extreme dehydration, ruptured pericarp and oozing. The browning of the pericarp of litchis is believed to be due to dehydration and degradation of anthocyanidin by polyphenol oxidase and peroxidase (Joasa et al., 2005). Dehydration, pitting and change in colouration was observed in the untreated tomatoes. There was also a significant loss in the firmness of tomatoes after 21 days. The deterioration index by 21 days of storage as assessed by visual inspection was 4 in the case of both untreated litchis and tomatoes (Table 1). Similar chilling injuries such as pitting, fungal infestation and uneven ripening have been reported in fruits of tropical origin such as tomatoes when refrigerated at temperatures lower than the critical temperature of 10 ^{0}C (Holderbaum, 2010).

Table 1: Deterioration index of tomatoes or litchis that were either untreated or treated with UV-C /HWT and stored for different time periods. 1= no damage, 2= mild damage, 3= moderate damage, and 4 = severe damage

Deterioration Index	DAY 0	Day 7	Day14	Day 21
TOMATO				
Control	1	2	3	4
UVC	1	1	2	2
HWT	1	1	2	2
LITCHI				
Control	1	2	4	4
UVC	1	2	2	2
HWT	1	2	2	2

Both antioxidant activities as well as total phenolic content decreased significantly with an increase in storage time duration. This has been observed in other phenolic-rich fruits and vegetables in which tissue disruption may facilitate the access of polyphenol oxidases and peroxidases to their substrates (Minatel *et al.*, 2017). The antioxidant activity of tomatoes decreased by more than 50% whereas TPC values decreased by 75% by 21 days post storage (Figures 2a & 2b).

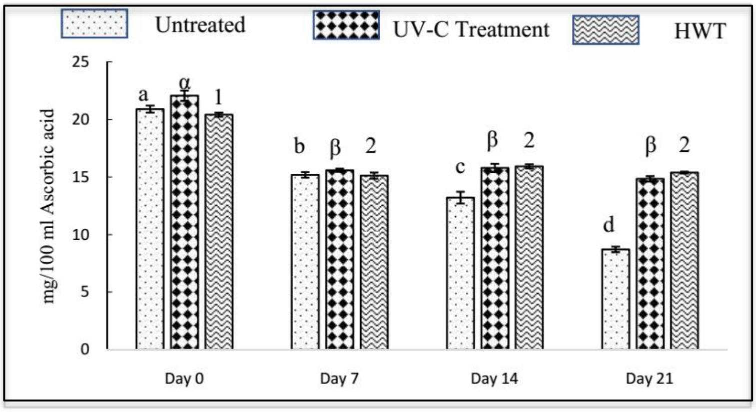

Figure 2a: Storage-induced changes in antioxidant activity as measured by FRAP assay of tomatoes that were untreated or underwent postharvest treatment. Results are expressed as mg/100 ml of AA. Values having significant difference ($p<0.0001$) are expressed by different superscripts

In the case of litchi, the decrease in antioxidant activity and TPC was not very acute. By day 21 of storage, 26% of antioxidant activity was lost and TPC was reduced by 49% (Figures 3a & 3b). Interestingly and importantly, there was no correlation between the morphological deterioration and antioxidant activity as well as TPC. Hence, the morphological deterioration cannot be used as an index of estimation of loss in nutritional values.

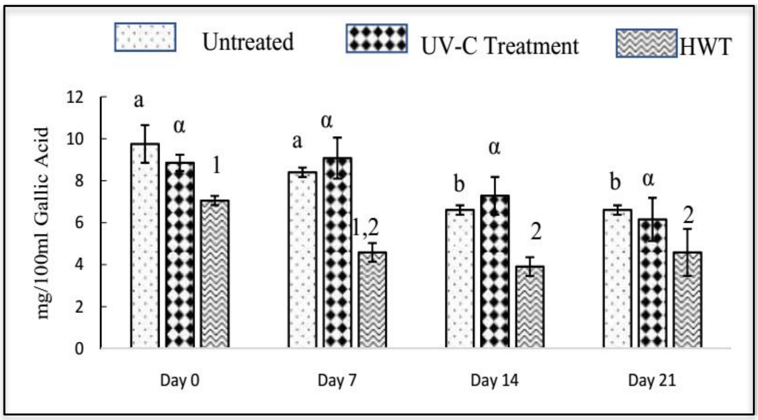

Figure 2b: Storage-induced changes in total phenolic content (TPC) of tomatoes that were untreated or underwent postharvest treatment. Results are expressed as mg/100 ml of GA. Values having significant difference ($p<0.0001$) are expressed by different superscripts

3.2. Effect of UV-C and heat shock treatment on tomatoes and litchis stored for different time duration

In case of tomatoes, HWT was unable to increase the shelf life as the deterioration index was equal to that of the untreated group. However, UV-C treatment was able to maintain the tomatoes in a better condition up to day 21 as evidenced by the deterioration index of 3 (Table 1). Litchis that were treated with heat shock or UV-C were in better condition up to day 21 as the deterioration index was 3, which was less than the untreated group (Table 1).

In tomatoes, treated with UV-C as well as heat shock, the decrease in antioxidant activities upon storage was much less as compared with untreated tomatoes (Figure 2a). In case of TPC, surprisingly, treatment with UV-C decreased the total phenolic content in the control group which however increased when stored for the different time duration (Figure 2b).

The decrease in TPC may be due to the effect of UV-C on phenylpropanoid pathway that is responsible for the synthesis of phenols as suggested by Ke and Saltveit (1989) or due to alteration of polyphenol oxidase activity responsible for oxidizing phenol by UV-C as shown by Kim and Jung (2011). However, with time, the increase in TPC value can be considered as a result of the adaptive mechanism. UV stress has been shown to promote the enzymatic activity of phenylalanine ammonia lyase which is a key enzyme in the phenylpropanoid pathway for the production of phenols (Ryals *et al.*, 1996). In litchis, both UV-C and heat shock treatment were capable of abrogating the storage-induced changes in antioxidant activities and TPC values. Both, antioxidant activity and TPC of the treated 21-days stored litchis were equivalent to control values (Figures 3a &b).

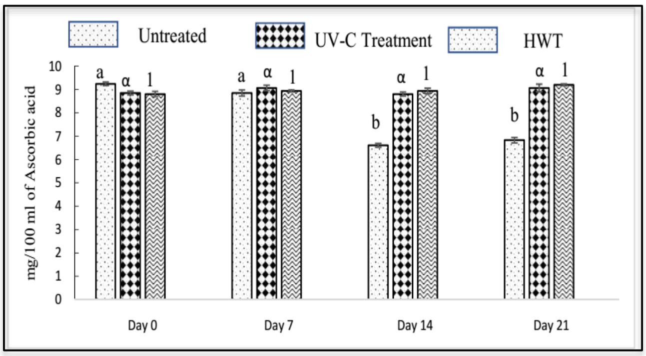

Figure 3a: Storage-induced changes in antioxidant activity as measured by FRAP assay of litchis that were untreated or underwent postharvest treatment. Results are expressed as mg/100 ml of AA. Values having significant difference ($p<0.0001$) are expressed by different superscripts.

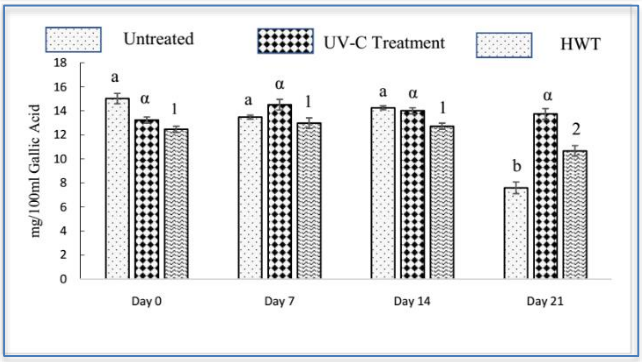

Figure 3b: Storage-induced changes in total phenolic content (TPC) of litchis that were untreated or underwent postharvest treatment. Results are expressed as mg/100 ml of GA. Values having significant difference ($p<0.0001$) are expressed by different superscripts.

Other chemical post-harvest techniques have been reported to increase the shelf life of litchi such as a combination of ascorbic acid and chitosan treatment (Sun *et al.*, 2010), and coating with aloe vera gel (Ali *et al.*, 2019). Both these treatments were effective in reducing pericarp browning and inhibiting loss of antioxidant properties. However, this is the first time that UV-C and heat shock treatment as an effective means of preserving the nutraceutical properties of litchis, is being reported.

4. CONCLUSION

Fresh fruits and vegetables are an important part of a balanced diet. Despite being the second largest producer of fruits and vegetables globally, India suffers huge economic losses due to spoilage during cold storage. The present study quantifies the spoilage and loss of antioxidant properties in litchis and tomatoes when stored in the refrigerator for different durations. Also, this study suggests that simple cost-effective postharvest techniques such as UV-C and HWT can go a long way in increasing the shelf life of the produce and reducing the loss of important antioxidants and total phenols. For the first time, the use of UV-C and HWT for increasing the shelf life of litchis has been explored.

5. CONFLICT OF INTEREST

None

6. SOURCE OF FUNDING

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