

Use of Additives to Reduce Browning, Microbial Load and Quality Loss of Kinnow Juice under Ambient Storage

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Abstract

Backgrounds/Objective: Preservation of Kinnow (*Citrus nobilis* × *Citrus deliciosa* L.) juice by using edible preservatives is needed to ensure nutritional security of common people. Suitability of preservation techniques to extent the storage life through maintenance of microbial load and nutritional strength up to desirable level is essential. **Methods/Statistical Analysis:** The study was conducted through analysis of TSS, acidity, Vitamin-C, and sugar while biological study was carried out through microbial load and browning. **Findings:** Kinnow fruit juice can be stored up to 30 days without loss of nutritional quality if 0.05% of Potassium metabisulphite or 0.05% sodium benzoate is used as preservatives whereas minimum and desirable browning (0.093) and microbial load (2.60×10^5) was reported when juice was treated with 0.1% KMS at 30 days of storage. **Application/Improvements:** The research outcome of present investigation suggests for storage of Kinnow fruit juice for 30 days with minimum quality loss by using 0.05% Potassium metabisulphite or sodium benzoate under ambient condition.

Keywords: Ambient Storage, Browning, Kinnow, Microbial Load, Preservatives, Shelf Life

1. Introduction

Kinnow (*Citrus nobilis* × *Citrus deliciosa* L.) is one of the important citrus fruits known not only for its beautiful appearance and pleasing flavor but also for its excellent food qualities, high nutritive value and high productivity. As per NHB (National Horticulture Board) database (2015) the mandarin cover largest area (3.3×10^5 ha) among all citrus species which is 4.6% of total area under fruit crops. The common mandarins that are under commercial cultivation include Kinnow, Nagpur, Khasi and Darjeeling. Among all mandarins, Kinnow accounts for highest production and productivity. In India, Punjab, Maharashtra, Karnataka, Tamil Nadu, M.P., Rajasthan, West Bengal, Himachal Pradesh and Haryana are commercial producers of Kinnow. Vitamin-C content of Kinnow juice is high with fair amounts of vitamins A and

B and significant amount of minerals including calcium, phosphorus and iron¹. In² has advocated that inclusion of citrus fruit juice in diet could prevent coronary diseases and asthma. In³ had reported the presence of antioxidant, anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activity and many bioactive compounds like ferrulic acid, hydrocinnamic acid, cyanidinglucoside, hisperidine, vitamin-C, carotenoid and naringin in citrus fruit juice. This is also in accordance with⁴. In⁵ reported the first commercial production of citrus seed oil from grape fruit seeds. Application of citrus seed oil in the preparation of fatty acid derivatives make it suitable for use in leather and textile industries.

As reported by⁶ natural juices have short shelf life even under refrigeration. Technological inefficiencies in processing and storage results in change in the composition of fruit or fruit juices. The organic acids present in fruits

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acts as natural preservatives for fruits and fruits juice. However, several disease outbreaks have been reported due to consumption of traditionally preserved juice. In⁷ reported that consumption of traditionally preserved apple cider resulted occurrence of hemolytic uremic syndrome in 1991 due to infection of *Escherichia coli* O157: H7 strain.

Fruit juice preservation is an economic business practice and the countries enriched with fruit resources are giving emphasis over establishment of storage and preservation systems to enhance shelf-life and quality of fruits so as to insure their availability during off-season⁸. The advent of heat sterilization, refrigerated storage and drying has given a great impetus to modern method of food preservation. Although many preservatives have been recommended for use in foods, sulphur dioxide and benzoic acid dominate the scene, and find wide spread applications. Due to these facts, the investigation was conducted out in order to determine the effect of preservatives and storage duration on the chemical constituents, non-enzymatic browning and microbial load of mandarin juice under ambient condition.

2. Materials and Methods

2.1 Site of Investigation

The study was conducted in Postharvest Laboratory of Department of Horticulture, Lovely Professional University, Phagwara, Punjab. The sub-region is characterized by hot dry sub-humid to semi-arid transition with dry summers and cool winters. The mean annual air temperature ranges from 24 to 26°C. The mean maximum summer (May to July) temperature ranges from 35 to 39.4°C rising to a maximum of 40°C in May to June. The mean winter (December to February) minimum temperature ranges from 4°C to 6°C dropping to a minimum of 3.7°C- 4.4°C during December and January. The sub-region receives annual mean rainfall ranging between 700-1000 mm covering 52-60 percent of mean annual PET (Potential Evapo Transpiration) ranging between 1300-1500 mm. The monsoon last from June end to September end covering 75-80 per cent of total annual rainfall.

2.2 Preparation of Fruit Samples

The Kinnow fruits of uniform size, disease free were picked randomly from all the four directions of the plants

with the help of secateurs at physiological maturity. The fruits were collected in plastic crates and shifted to the Postharvest laboratory, where the fruits were sorted, washed and graded. Thereafter, fruits were divided into requisite lot for further handling.

2.3 Extraction of Juice and Bottling

The fruits were cut into two halves and juice was extracted by automatic citrus juice extractor. The whole of the juice was then filtered to obtain a clear juice. The treated juice was then poured into hot well sterilized crown glass bottles of 200 ml capacity and corked air tight. Where pasteurization was required, the filled bottles were pasteurized in boiling water bath for 30 minutes at 63°C temperature of the product. All the juice filled bottles were stored under ambient condition (8-29°C) and the different parameters were studied at 0, 15, 30 and 45 days of storage.

2.4 Experimental Details

The study was carried out by using different preservatives treatments. There were eight treatments, each having three replication. The treatments were: T₁ (Control or untreated juice), T₂ (Pasteurization at 63 °C for 30 minutes), T₃ (Heating the juice to 85°C), T₄ (Sodium benzoate @ 0.05%), T₅ (Sodium benzoate @ 0.1%), T₆ (Potassium meta-bi-sulphite @ 0.05%), T₇ (Potassium meta-bi-sulphite @ 0.1%) and T₈ (Potassium meta-bi-sulphite @ 0.05% and Sodium benzoate @ 0.05%).

2.5 Observations Recorded

The Total Soluble Solids (TSS) content of the juice was determined at ambient temperature with the help of hand refractometer and expressed in degree brix (°B)⁹. The ascorbic acid content of fruit juice was estimated with the help of 3% metaphosphoric acid and 2, 6-dichlorophenolindophenol dye and expressed in mg/100ml of fruit juice⁹. The pH of the fruit juice was directly measured on the electronic pH meter. Total sugar and reducing sugar in all products were estimated by Lane and Eynon's method as reported by¹⁰. Browning was observed by taking transmittance of the solution in spectronic -20. The samples for browning were centrifuged for 15 minutes at 4000 rpm. After taking 10 ml of centrifuged, 15 ml of alcohol was added to make 60 per cent aqueous solution and kept for half an hour. The solution was filtered through Whatman (No.-1) filter paper to obtain a clear solution and the absorbance was measured at 440 nm.

The higher value of absorbance was considered as the extent of non-enzymatic browning. Microbial contamination was studied by a series of dilution and spread plate method as described by¹⁰.

2.6 Statistical Analysis

The analysis of data was carried out through CRD (Completely Randomized Design). The testing of significance of difference between the treatment means was done with the help of Critical Difference (CD) at 5% level of significance. The overall significant differences between the treatments were determined by F-test.

3. Results and Discussion

3.1 Total Soluble Solid (TSS)

The data pertaining to change in TSS of Kinnow juice stored under ambient condition has been represented through Figure 1. The TSS was reported to get increased with increase in storage duration and the maximum TSS was reported on 45 day of storage which may be due to greater hydrolysis of poly-saccharides in mono-saccharides and oligo-saccharides during storage. This trend of increasing TSS during storage has also been reported by¹¹ in blended Kinnow juice under ambient storage condition according to¹² in citrus juice and according to¹³ in

blended mango juice. The highest and most significant increase in TSS was reported with T₆ [0.05% KMS (11.80 at 0 day to 13.27° brix at 45 day)] followed by T₈ [0.05% KMS+ 0.05% SB (11.47 at 0 day to 13.20° brix at 45 day)] and T₇ [0.1% KMS (11.67 at 0 day to 13.13° brix at 45 day)] in comparison to T₁ [control (11.53 at 0 day to 12.0° brix at 45 day)] under ambient storage condition. The addition of KMS had significantly improved TSS level of stored fruit juice which may be due to prevalence of acidic medium created by sulphurous acid, formed by release of SO₂ from KMS. This may have resulted in the acid hydrolysis of complex insoluble compounds into simple soluble ones. The effect of preservatives on increase in TSS is in conformity with¹⁴ in apple pulp storage and according to¹⁵ in orange juice. Interestingly, 0.1% KMS has resulted in short increase in TSS in comparison to 0.05% KMS and its combination with sodium benzoate which may be due to inhibitory effect of high concentration of KMS.

3.2 Ascorbic Acid

The vitamin C content has been reported to get significantly reduced during storage of Kinnow juice under ambient storage condition irrespective to the treatments (Figure 2). About 30-45% of ascorbic acid was found to be degraded for different treatments (Figure 3), which may be due to effect of light, interaction with metallic ions and prevailing temperature as reported by¹⁶ in blanched and

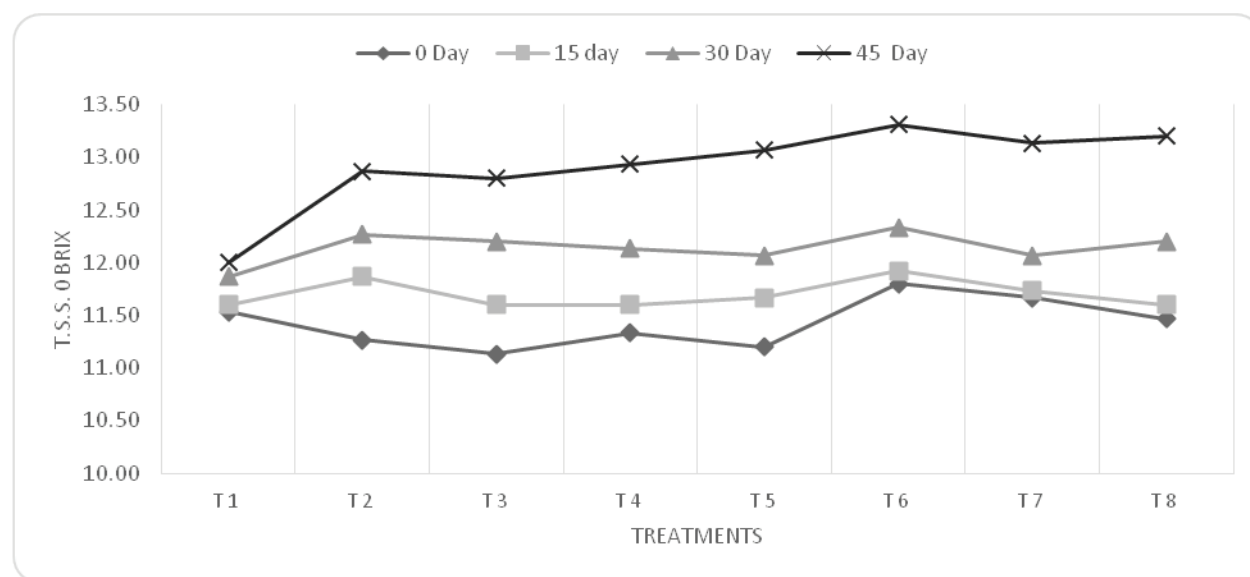


Figure 1. Variation in TSS of Kinnow juice as function of preservatives and storage duration.

unblanched aloe juice supplemented Kinnow nectar and confirmed by¹⁷ in citrus juice; according to¹⁸ in fruit juice; according to¹⁹ for different fruit beverages and according to²⁰ in cashew apple juice. In²¹ has also reported 29-41% loss of vitamins in fruit juice after 4 month of storage and is in accordance with findings of²². Furthermore, the decomposition of vitamin C follows first order kinetics and so is significantly influenced by storage time as revealed by²³. The addition of KMS in preserved Kinnow

juice has resulted significant retention of ascorbic acid in comparison to the heat treated (T_3), pasteurized (T_2) and untreated (T_1) Kinnow juice. Under ambient condition the significantly minimum (10.23, 19.32 and 27.25 %) loss of ascorbic acid was reported with T_6 (0.05% KMS) followed by T_8 [0.05% KMS + 0.05% SB (11.76, 21.18 and 29.41 %)] and by T_7 [0.1% KMS (12.9, 21.51 and 31.18%)] at 15, 30 and 45 days of storage while the maximum (24.68, 37.39 and 44.6 %) loss was reported

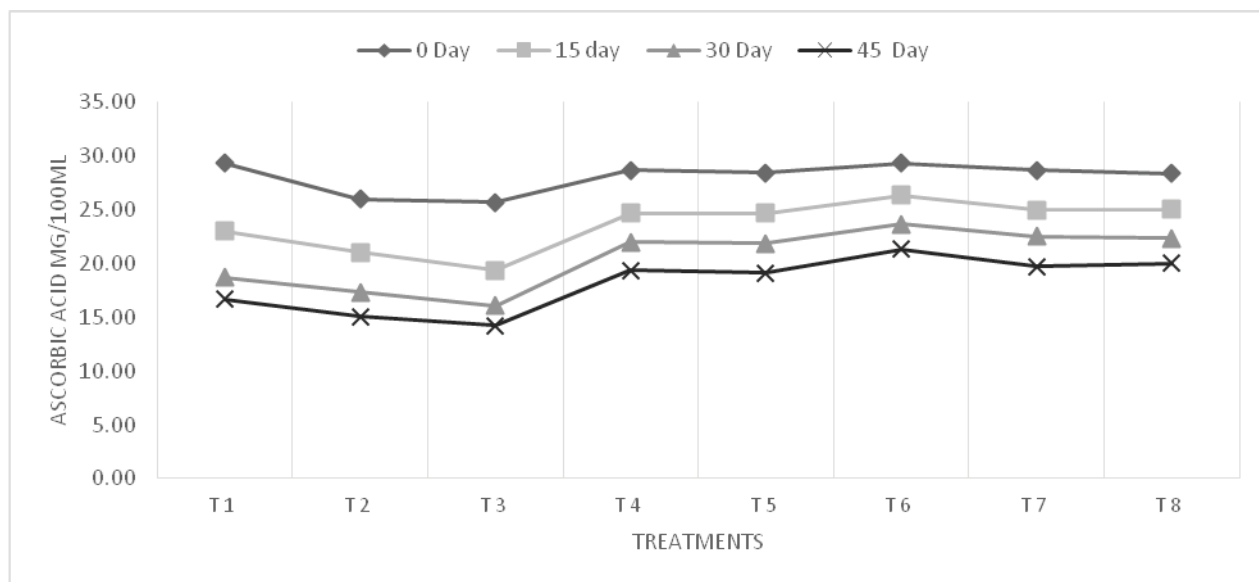


Figure 2. Variation in ascorbic acid of Kinnow juice as function of preservatives and storage duration.

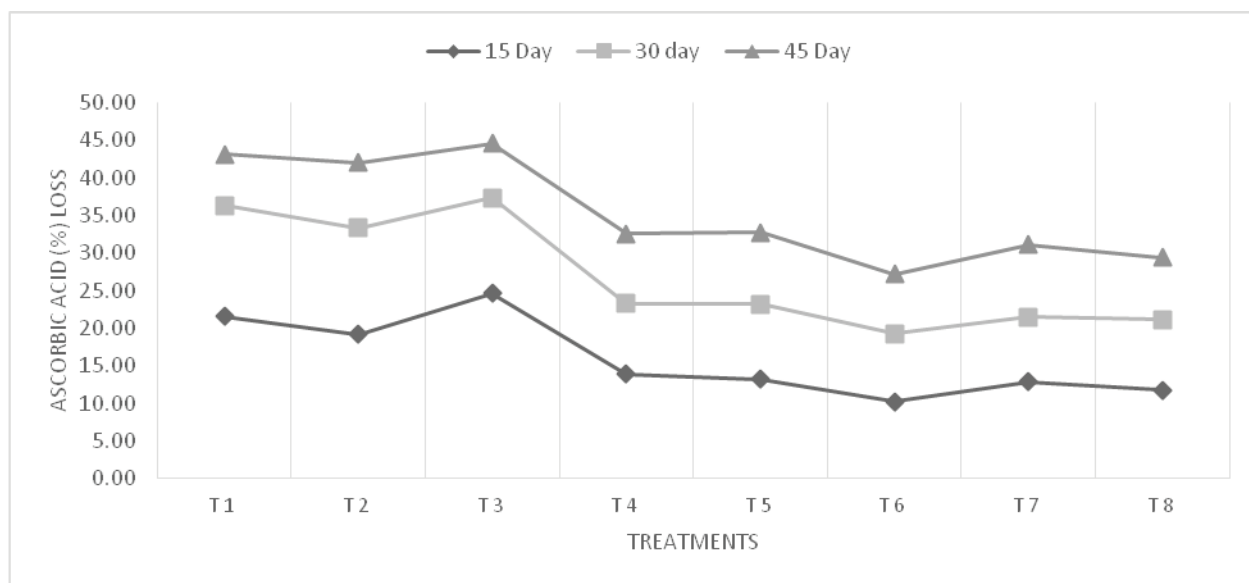


Figure 3. Ascorbic acid loss during ambient storage of Kinnow juice.

with T_3 (heat processed Kinnow juice). The effectiveness of KMS to prevent the degradation of vitamin C is due to its potential to release SO_2 which act as anti-microbial agent and stabilize ascorbic acid as confirmed by²³. The effect of KMS to preserve the vitamin C content of fruit juice has also been confirmed by^{24,25} for storage of leafy vegetables, according to¹⁹ for Kinnow juice and according to²⁶ in aonla juice storage. The heating and pasteurization significantly reduce vitamin C retention of Kinnow juice which can be confirmed by the finding of²⁶ in aonla juice, according to^{12,27} for citrus juice.

3.3 Titrable Acidity

It has been observed that the total acidity of Kinnow juice decreased with the storage duration for all treatments (Figure 4). A significant fall in acidity level, measured in form of citric acid, have been reported for all treatments which was resulted due to interaction effect of organic components of the juice with temperature and enzymatic action. The finding is in conformity with²⁸ for six month storage of bael RTS, according to²⁹ for 74 day storage of Kinnow juice, according to³⁰ for storage of mosambi fruits and according to³¹ for storage of Kinnow orange juice. The minimum loss of acidity and maximum acid content was observed in T_6 (0.05% KMS) which was 0.78% under ambient condition at 45 days of storage. It was followed by T_8 (0.05% KMS + 0.05% SB) which was 0.76% at 45 days of storage. The minimum total acidity was reported

in T_1 (control) which was 0.61% under ambient storage condition at 45 days of storage. The finding confirmed that there is a significant effect of using KMS³² and SB¹² preservatives in reducing loss of acidity during storage which may be due to alteration in metabolism and enzymatic activity.

3.4 pH

The observation recorded on the impact of storage duration on pH content of Kinnow juice treated with different preservatives showed significant impact (Figure 5). It is revealed that the pH of Kinnow juice was significantly increased during all storage periods under ambient, refrigerator and incubator condition. The maximum pH was reported at 45 days. The increase in pH during storage was accompanied with decrease in acidity of juice and is in conformity with the findings of³³ who had mentioned the significant and high pH value of mango and peach juice concentrates at 30 days of storage. The possible reason for increase in pH with prolonged storage of Kinnow juice may be due to acid hydrolysis of the poly-saccharides into mono-saccharides and di-saccharides, which are responsible for increase in sweetness and decrease in sourness. The above findings are also justified with conclusion drawn by³⁴. The minimum and significant increase in pH was reported with T_6 [0.05%KMS (3.38-3.61)] followed by T_8 [0.05%KMS + 0.05% SB (3.38-3.62)] under ambient condition at 45 day of storage. The highest

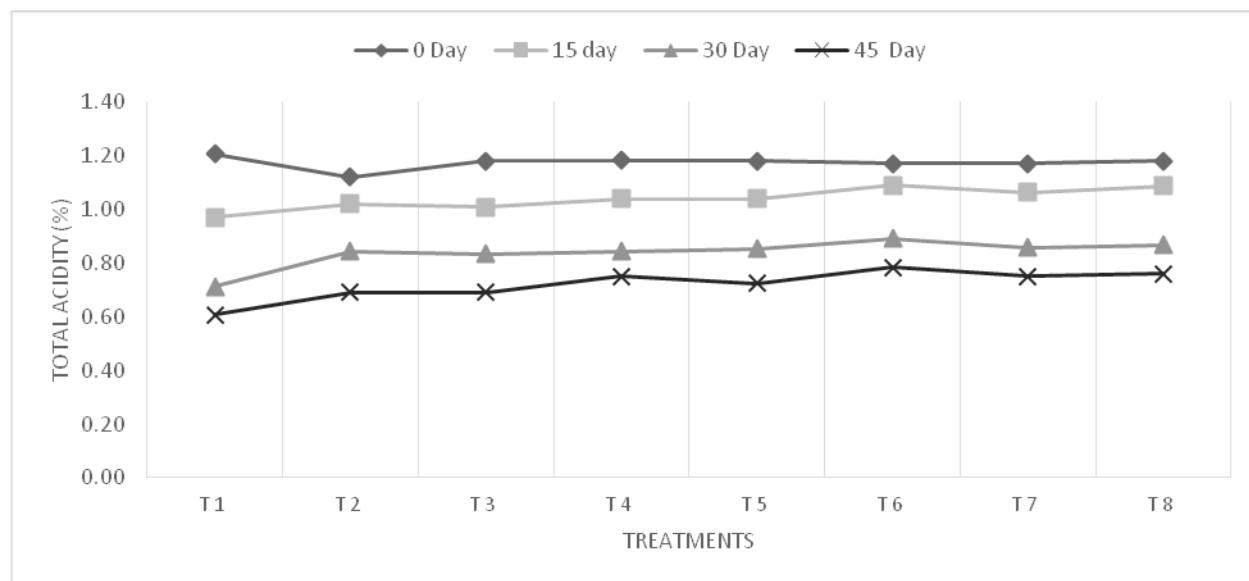


Figure 4. Variation in titrable acidity of Kinnow juice as a function of preservatives and storage duration.

increase in pH was reported with T_1 [control (3.37-3.81)], T_2 [pasteurized Kinnow juice (3.41-3.71)] and T_3 [heat treated (3.39-3.69)] under ambient condition at 45 days of storage. Present findings are in conformity with the investigation of³⁵ who revealed that there is synergistic effect of pasteurization, acidification and treatment with preservatives to extend the shelf-life of soursoop juice.

3.5 Reducing Sugar

The observations recorded on reducing sugar content of Kinnow juice revealed that the reducing sugar increased during storage for each treatment (Figure 6) which may be result of gradual conversion of non-reducing sugar, non-sugar carbohydrates and acids into reducing sugar. This is in conformity with the findings of³⁶ in cashew

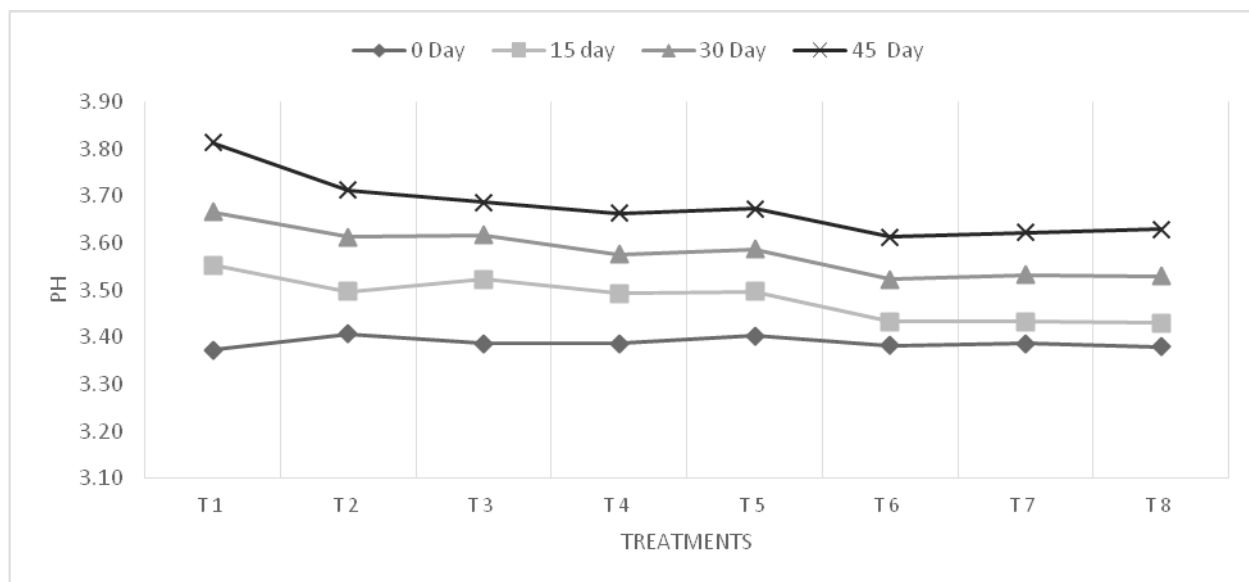


Figure 5. Variation in pH of Kinnow juice as a function of preservatives and ambient storage.

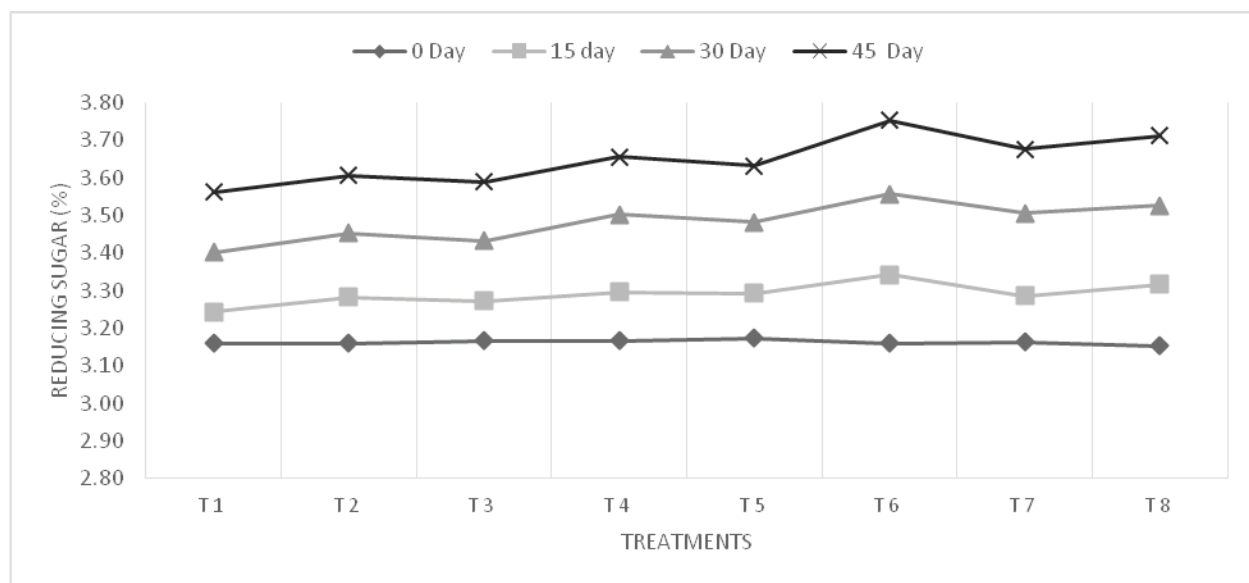


Figure 6. Variation in reducing sugar of Kinnow juice as a function of preservatives and ambient storage.

apple juice, according to³⁷ in gooseberry juice, according to²⁰ in cashew apple juice blended with coconut and according to¹⁷. The significant increase in reducing sugar content was reported in T_6 (3.16-3.75%) followed by T_8 (3.15-3.71%) and T_7 (3.16-3.68%) as compare to T_1 [control (3.16-3.56%)] under ambient storage condition. The most significant increase, by addition of KMS, in reducing sugar during ambient storage can be attributed to the higher acidity caused by formation of sulphurous acid due to dissolution of SO_2 released from KMS in juice, which may have resulted in the rapid inversion of non-reducing sugar into the reducing sugar. However, an interesting fact was observed with T_7 (0.1% KMS) which showed lower increase in reducing sugar in comparison to T_6 (0.05% KMS) which may be due to inactivation of proteolytic enzymes with high concentration of SO_2 . Furthermore, 0.1% KMS is also not suitable in terms of food safety. The similar finding were reported by³⁸ for storage of bael fruit pulp and squash according to¹⁹, according to^{37,39}, for aonla juice and according to³² in mandarin juice. The effect of sodium benzoate as preservative to increase reducing sugar can be justified by reports of⁴⁰ in storage of mango pulp and according to¹⁵ in storage of orange juice.

3.6 Total Sugar

The total sugar (Figure 7) content also showed similar trends as of reducing sugar which may be due to gradual hydrolysis of non-sugar carbohydrates or complex food

materials into simple sugar. This is in conformity with⁴¹ for bael-papaya blended RTS beverage and according to¹³ in mixed fruit juice RTS. The maximum increase in total sugar was reported with T_6 (7.52-8.55%) which was at par with T_8 (7.51-8.52 %) and T_7 (7.50 – 8.49 %) while, the minimum total sugar was reported with T_1 (7.51-8.35 %). The greater increase in total sugar due to KMS may be due to release of SO_2 , which created acidic medium to induce acid hydrolysis and rapid inversion of poly-saccharides to sugars. This is in conformity with³² in mandarin, according to¹⁷ for citrus juice and according to³⁷ in blended aonla juice.

3.7 Non-Enzymatic Browning

Browning is a result of accelerated non-enzymatic browning reaction at higher temperature as conformed by^{11,42,43}. Most of the preservatives have significantly reduce the rate of browning of fruit juice in comparison to control and the pasteurized juice. The significantly least browning was reported in T_6 [0.05% KMS (0.086-0.093)] followed by T_8 [0.05% KMS + 0.05% sodium benzoate (0.0862-0.094)] and T_7 [0.1% KMS (0.086-0.098)] under ambient condition while, the heat treated, pasteurized and untreated juice suffered greater loss in quality and high degree of browning after 45 days (Figure 8). The inhibitory effect of SO_2 released from KMS may be responsible for slowing down of non-enzymatic browning reaction and the retention of natural color of the juice. This can be confirmed

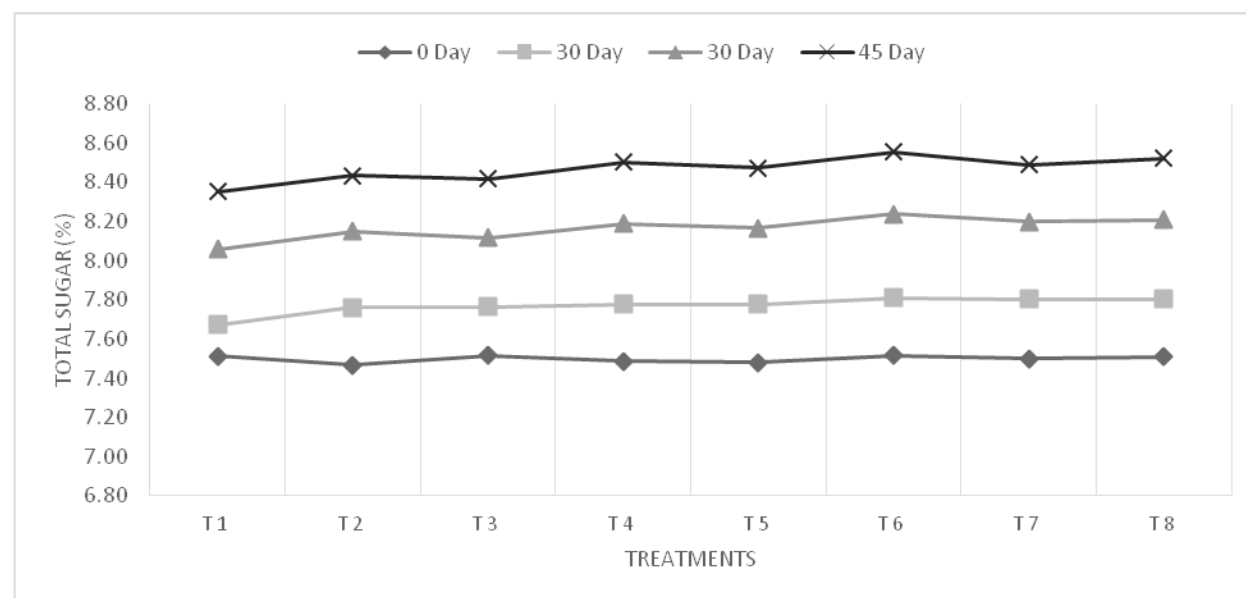


Figure 7. Variation in total sugar of Kinnow juice as function of preservatives and ambient storage.

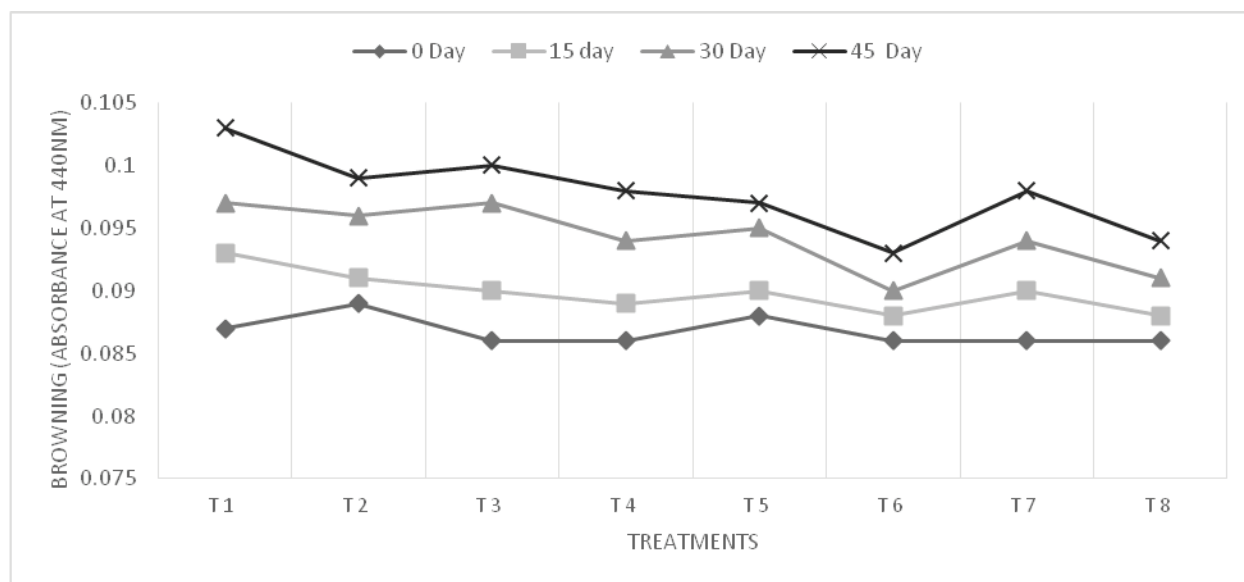


Figure 8. Variation in absorbance by Kinnow juice at 440nm wavelength.

by the report of^{19,44}. According to⁴⁵ has also observed similar result for mango pulp in storage and is in conformity with⁴⁶ in aonla juice and according to⁴⁷ for blended Kinnow juice.

3.8 Microbial Contamination

The existence of microbial contamination in the processed products is the function of the quality of raw materials, processing instruments, storage or processing atmosphere, packaging materials and the individuals employed in the processing process. According to⁴⁸ reported that the approximate fungal count in most of the processed products is in order of 10^3 to 10^5 . The lower order of microbial count is indicator of the safe and consumable fruit juice while the high order ($>10^5$) is not safe for consumption. The observation recorded on microbial contamination being represented through Table 1. At ambient temperature the microbial count was observed to get increased for all treatments and remained within safe range up to 30 days of storage for some of the treatments (T_4 to T_8) but at 45 days not a single treatment retained the safe order of microbial load. The present report is in conformity with⁴⁹⁻⁵¹ for papaya juice. The minimum (1.70×10^4 and 2.60×10^5) microbial load was reported with T_7 (KMS 0.1% treated juice) which was significantly better than T_8 [KMS 0.05% +SB 0.05% (2.51×10^4 and 2.89×10^5)], T_6 [KMS 0.05% (3.30×10^4 and 3.73×10^5)], T_5 [SB 0.1% (3.36×10^4

Table 1. Effect of different treatments on microbial counts of stored Kinnow juice under different storage conditions

Treatments	Ambient Temperature			
Days	0 day	15 days	30 days	45 days
T_1	1.40×10^3	4.20×10^4	2.50×10^6	1.70×10^8
T_2	1.40×10^3	4.30×10^4	1.93×10^6	1.50×10^8
T_3	1.40×10^3	4.50×10^4	1.01×10^6	1.08×10^8
T_4	1.40×10^3	3.60×10^4	4.70×10^5	2.91×10^6
T_5	1.40×10^3	3.36×10^4	4.01×10^5	2.60×10^6
T_6	1.40×10^3	3.30×10^4	3.73×10^5	1.70×10^6
T_7	1.40×10^3	1.70×10^4	2.60×10^5	1.01×10^6
T_8	1.40×10^3	2.51×10^4	2.89×10^5	1.24×10^6

and 4.01×10^5) and T_4 [SB 0.05% (3.60×10^4 and 4.70×10^5)] when observed at 15 and 30 days, respectively under ambient storage while the Kinnow juice stored without preservatives (T_1 , T_2 and T_3) showed excessive microbial load at 30 days which justified that Kinnow juice is not acceptable after 15days when stored at ambient condition without adding any preservatives. There was a significant effect of KMS in inhibiting the microbial multiplication in anaerobic environment. This is in conformity with¹³ for preservation of spiced RTS beverages, according to^{52,53} while, studying microbial growth on the mango pulp,

according to^{54,55} while, studying preservation of fruit pulp. In³⁵ reported inhibitory effect of SB on microbial growth in sour sop and can be further confirmed with the findings of⁵⁶. Increase in microbial count was also reported by⁵⁷ during storage of rabri supplemented with different types of milk when stored at 5°C.

4. Conclusion

Our findings emphasize that preservatives like potassium metabisulphite and/or sodium benzoate in appropriate concentration can be used to enhance shelf life of Kinnow juice under ambient storage condition. The chemical and microbial quality parameters reflect that the nutritive value of juice is also retained up to acceptable level even at 30 days of storage. Further research under refrigerated storage may result in enhancement of shelf life and retention of even high nutritive value which should be investigated.

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