

## Impact of thermo-sonication on antioxidant potential in juices of selected citrus varieties

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### Abstract

Citrus is known as a good source of valuable bioactive components and vitamins. In fruit juices, thermal treatments such as pasteurization are usually used for inactivation of enzymes and for destruction of microorganisms. Thermo-sonication is combination of heat (37 to 75 °C) with ultra-sonication treatment has high efficiency for inactivation of microbes and enzymes. The present study aims to develop a new processing technique for citrus juice to improve its functional properties. Variety Succari excelled in terms of average fruit diameter and weight and the maximum number of segments found in Feutrell's Early. The highest acidity value was observed in T0 (0.251±0.008 %) and the lowest in T1 (0.147±0.002 %) juice of Kinnow. Acidity value was decreased by application of pasteurization. Total phenolic contents of Musambi juice showed significant increase in thermo-sonicated and sonicated juice in comparison to non-sonicated juice. The increase was high in T4 (458.89±6.72 µg/mL of GAE) and T5 (450.37±9.83 µg/mL of GAE). Antioxidant activity showed significant increase in thermo-sonicated and sonicated juice. The increase was high in T5 (1,103.20±9.94 µg/mL of ascorbic acid equivalent) and T5 (1,110.74±23.67 µg/mL of AAE) in juice of Kinnow. Total plate count showed significant reduction in thermo-sonicated (T4) and in combined treatment of chemically preserved and pasteurized (T3) juice in comparison to sonicated (T5) and non-sonicated (T1 and T2) juice. The minimum decrease of total plate count was found in juice of Feutrell's Early while maximum reduction was checked in the juice of Musambi during storage.

**Keywords:** Citrus; physical properties; acidity; total phenols; anti-oxidant; AAE (Ascorbic acid equivalent); GAE (Gallic acid equivalent)

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## Introduction

Fruits are healthy food which provides minerals, vitamins and dietary fiber (Rocha and Morais, 2003). The genus citrus belonging to the family *Rutaceae* consists of almost 40 types and is considered as a world's important crop and consumed in both of its form, as fresh fruit and as well as juice, because it has valuable nutritional value and unique flavor. Some juices are recommended to be the part of human diet because of having functional components like natural antioxidants and vitamin C which has the capability to inhibit the formation of different clinical circumstances like heart diseases and certain cancers (Khosa et al., 2011). Citrus fruit juices have significant quantity of phenolics, carotenoids and flavonoids and their calculable variations in the proportions occurs because of agro-climatic conditions and origins (Khosa et al., 2011). Citrus includes anti-oxidants that minimizes the risk of numerous diseases like cardiovascular, certain cancers and arteriosclerosis (Louche et al., 2000). The juice of citrus comprises of phenolic compounds, minerals, amino acids, carbohydrates and proteins etc. Vitamins, minerals and other food components in the citrus fruit has capability to improve weak health rapidly (Karimi et al., 2012).

During marketing and transportation, the perishability of citrus juice can be enhanced by pasteurization. Carotenoids are considered as heat stable during cooking, canning and blanching (Borenstein and Bunnell, 1966), but carotenoid stability differs significantly in foods (Purcell et al., 1969). Previously, it was thought that pasteurization had no effect on the constituents of carotenoids. But during a thermal processing study on fruit and vegetables including citrus juice, conducted by (Lessin et al., 1997) showed a comparatively excessive loss (up to 36 %) of carotenoids (b-cryptoxanthin, a and b carotene) and pro-vitamin A. The juice of citrus is one of the most generally consumed drinks because of its health benefits, unique taste and aroma (Ren et al., 2015).

In thermal processing techniques heat is commonly used for the inactivation of enzymes and also to kill the microbes. Over the last few years, heat processing methods were preferred with the combination of new technological advances. But excessive heat causes different chemical and physical changes which alter the sensory characteristics of food products and minimize the bioavailability of nutrients and their properties (Pereira and Vicente, 2010).

Nowadays, different non-thermal technologies are accessible to achieve the requirement of healthy and natural fruit juices with negligible effect of its natural organoleptic and nutritious characteristics. Common thermal processing methods for fruit juices effects in some nutritious component's loss such as carotenoids (Fратиanni et al., 2010) and flavonoids (Iguar et al., 2011). There might be the chances of off flavors, darker color and excessive suspended solids (Rutledge, 1996).

Ultrasound technique may also be used for the processing of fruit juices due to several benefits regarding the quality of fruit juices, reduced energy consumption, decreasing processing time, improving shelf life and increased efficiency. Ultrasound presented valuable properties in foodstuff preservation and processing with less working and maintenance costs, less processing times, greater product yields, enhanced quality characteristics and reduction of pathogens (Patist and Bates, 2008). It develops food safety and quality and have chances for developing new products with distinctive properties (Awad et al., 2012). The present project is planned to explore the impact of thermal and non-thermal processing techniques on functional properties of different citrus juices during storage.

## Material and Methods

### Purchase of fruits and chemicals

All the reagents and chemicals were purchased from Fluka Chemical Co. (Buchs, Switzerland) and Merck (Darmstadt, Germany). Citrus varieties were collected from the Citrus Research Institute, Risala No. 5. Sargodha, Pakistan. The research work was carried out at Institute of Food Science and Nutrition, University of Sargodha, Sargodha. Physicochemical characteristics of different varieties of citrus (Musambi, Succari, Feutrell's Early & Kinnow) were studied. Fruits of each cultivar were picked at the optimum time of maturity. The samples of fruit were collected from all sides of the tree i.e. from top and inside the canopy. Variation in plants and at the farm was also considered.

### Physical analysis of fruits

The sample of 20 fruits were taken to determine the quality characteristics at the optimum time of maturity. Fruit weight (g) was measured by using a digital calibrated scale. The number of segments was counted manually (Cheng, 2008).



### **Preparation of juice**

After weighing of each fruit, rotary citrus squeezer (MJ-M176P, Panasonic Manufacturing, Berhad, Malaysia) was used to extract the juice. The juice was filtered over 0.8 mm pore size sieve. Juice of each variety (100 mL) was separated into two equal parts (50 mL each) for further analysis.

### **Juice %**

Following expression was used to calculate the juice percentage (Khan et al., 2004).

$$\text{Juice \%} = \text{Juice weight (g)} / \text{Fruit weight (g)} \times 100$$

### **Chemical preservation**

For chemical preservation, the citrus juice was chemically treated by potassium meta-bisulphite (KMS) at the rate of 750 ppm.

### **Pasteurization of juice**

Pasteurization was done by the method of Dede et al. (2007). Heat treatment given to juice samples (bottles) in water bath at 81 °C for 90 sec for pasteurization (industrial pasteurization application) (Scalzo et al., 2004). During pasteurization, bottles were continuously stirred for proper heat transfer. Bottles were taken out when the required temperature achieved which was measured by digital temperature probe and then instantly cooled in an ice bath. All measurements and experiments were replicated thrice on separate days.

### **Ultrasound processing of juice**

The juice obtained from each variety was subjected to ultra-sonication process. The ultrasonic processor (UP400S, Hielscher Ultrasonics GmnH Hielscher USA, Inc.) with 0.5-inch probe was used for sonication. The juice was subjected to ultrasound treatment (pulse duration of 5 s on and 5 s off, amplitude level of 80 % and 20 kHz frequency) for 4 min at 20 ° C. The sonication was performed immediately after extraction of fresh juice. The analysis was done in dark. The fresh sample without sonication was used as a control. All juice samples (control or sonicated) were kept at 4 °C for more experiments.

### **Chemical analysis of juice**

#### **Total titratable acidity**

Titratable acidity was determined by titration method with standardized 0.1N NaOH up to pH 8.2.

Phenolphthalein as an indicator was used to check the persistent pink color using known volumes of a representative sample of juice. The results were expressed as grams of citric acid per 100 mL of juice (AOAC, 2012).

$$\text{Acidity \% (as citric acid)} = \text{Titer value (mL)} \times 0.0064 / 10 \times 100$$

### **Total phenolic contents**

Spectrophotometer (using Folin-Ciocalteu reagent with some modifications) was used for the determination of total phenolic contents (Singleton et al., 1999). Juice sample of known concentration (0.5 mL) was mixed with 1mL of 10 % Folin–Ciocalteu reagent. The blend was blended well and left it for 6 min. Then 2 mL of sodium carbonate solution (20 %) was added to that blend. The total phenolic contents were determined at 760 nm using spectrophotometer after incubation for 60 min at 30 °C. For calibration curve, standard solution was prepared by using Gallic acid. The results were shown as µg of gallic acid equivalents (GAE) per milligram of sample.

### **Total antioxidant activity**

For antioxidant capacity determination, citrus fruit juices of all varieties were determined by the technique defined by (Prieto et al., 1999). 0.4 mL of sample was mixed with 4 mL of reagent solution (0.6 M Sulfuric Acid + 28 mM Sodium Phosphate + 4 mM Ammonium Molybdate). The absorbance was measured at 695 nm after incubating for 95 min at 90 °C. Blank solution with reagent and methanol was run and absorbance was measured at the same wavelength. The results were expressed as µg ascorbic acid equivalent (AAE)/mL sample. All experiments were performed in triplicates.

### **Microbiological analysis**

The citrus juices after different treatments were stored in refrigeration (4±1 °C) for three months. Juice samples were analyzed for total plate count to check the shelf stability of the juices after every 1.5 month (Rockville and Latimer, 2016). 1 mL sample (citrus juice) was taken into test tube by sterile pipette and 9 mL saline solution was added for making dilutions. Decimal dilution was formed by shifting 1 mL of previous dilution to 9 mL. 15 mL of media (pre-cooled to 45 °C) was shifted in plates within 15 minutes of time and allowed to solidify. Dilutions were shifted in control plates then mixed sample dilutions and agar



medium. Petri dishes were incubated at 35 °C for 48 hours. All colonies were calculated between 30 and 300 colonies and multiplied by dilution factor.

### Statistical analysis

The data obtained from chemical assay was subjected to analysis of variance technique and mean values±SD were calculated by LSD test at 5 % level of significance. The statistical analyses were performed using Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA).

## Results and Discussion

### Physical properties of citrus varieties

Citrus variety Succari excelled in terms of average fruit diameter 73.46±1.13 mm and fruit weight 212.95±9.30 g followed by Kinnow 65.05±1.07 mm, 152.40±7.83 g. The minimum fruit diameter and weight were observed in Musambi 61.78±0.66mm, 127.45±3.45g respectively, and in Feutrell’s Early 61.49±0.71 mm, 114.25±3.75 g (Table 01).

The maximum number of segments found in Feutrell’s Early 11.20±0.22 and Succari 10.75±0.22 followed by Kinnow 10.40±0.18 while minimum number of segments were recorded in Musambi 10.10±0.16. The maximum juice 51.5 % was observed in Succari while the minimum juice 49.6 % was obtained by Musambi. Qureshi et al. (2020) studied the characteristics of indigenous varieties of mango (*Mangifera indica* L.). They found significant differences among varieties of mango (Sindhri, Anwar Ratol, Langra, Gulab Khas, Chaunsa, Sunehri, Dosehri and Desi). They found the maximum fruit weight in Sindhri 407.6 g and Chaunsa 255.9 g while minimum fruit weight in Desi and Anwar Ratol. The maximum pulp 73.28 % and edible to non-edible ratio 4.83 was observed in variety Sindhri. They described that some varieties of mango had high quantity of peel while some had low peel weight.

### Chemical analysis of citrus juices

Citrus Juices was stored in refrigerator at 4±1 °C and analyzed for acidity.

### Acidity of citrus juices

Acidity showed (Table-2) significant rise in all the samples (thermo-sonicated and sonicated juice and non-sonicated juice). T0 (control) showed the highest

value of acidity and T1 (chemically treated) showed the lowest value of acidity among all the citrus juices but the effect was significant among treatments. The highest acidity value was observed in T0 0.251±0.008 % and lowest value in T1 0.147±0.002 % in the juice of Kinnow (Table-3 and Figure-1). The sonication alone has high acidity value T5: 0.082±0.004 % as compare to thermos-sonication T4 0.081±0.002 % in the juice of Musambi (Table 3). Acidity value decreases by the implementation of pasteurization T3 0.074±0.002 % as compare to control T0 0.088±0.008 % (Table-3). Similar trend in acidity was observed in juices of other citrus varieties.

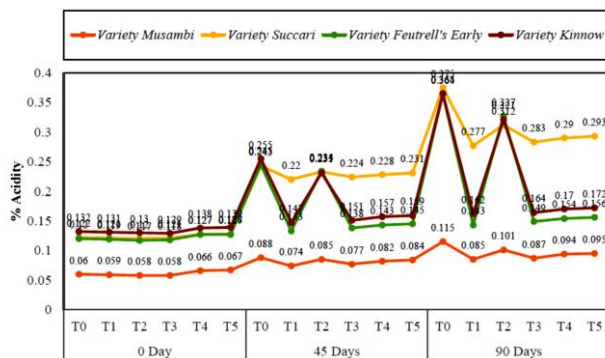


Figure-1. Comparison of means (Treatment x Days x Variety) for acidity (%)

The minimum increase of acidity was in the juice of Succari while the maximum increase was in the juice of Musambi during storage (Table-2). The acidity value of juices of Musambi, Succari, Feutrell’s Early and Kinnow increased significantly during storage of 90 days. The acidity gradually increased with the passage of time. The lowest value of acidity in fresh Musambi juice 0.062±0.003 % was found at start of storage. However, it increased significantly at 90 days of storage 0.100±0.005 % (Table-2). The increase in the acidity might be due to rupturing of cell wall and decrease in the pH.

Qureshi et al. (2020) have checked the influence of ultrasound on the functional characteristics of indigenous varieties of mango (*Mangifera indica* L.). They ultrasonic activity on acidity of mango pulp was found prominent difference. They found highest 1.36 acidity in the pulp (without US treatment) from Desi however pulp from Chaunsa displayed the 0.26 lowest value. They demonstrated that acidity of pulp was increased significantly after 4 min of US treatment whereas additional treatment altered the values non-significantly.

**Table-A: Treatment plan**

Citrus Variety		Control Treatment	Chemical Treatment (KMS 0.75g/1000 mL)	Thermal Treatments			Non-Thermal Treatments
				Pasteurization (81 °C for 90 s)	Chemical & Pasteurization (81 °C for 90 s + 0.75 g/1000 mL)	Thermo-Sonication (81 °C for 90 s + 04 min US)	Ultrasound (04 min)
Common Oranges	Musambi	T0	T1	T2	T3	T4	T5
	Succari						
Mandarins	Feutrell's Early						
	Kinnow						

**Table-1: Physical analysis of different citrus varieties (Means±SE) for fruit diameter (mm), fruit wt. (g), no. of segments and juice (ml).**

Variety	Musambi	Succari	Feutrell's Early	Kinnow	Means
Fruit Diameter (mm)	61.78±0.66 <sup>D</sup>	73.46±1.13 <sup>A</sup>	61.49±0.71 <sup>C</sup>	65.05±1.07 <sup>B</sup>	65.44±0.78
Fruit Wt. (g)	127.45±3.45 <sup>C</sup>	212.95±9.30 <sup>A</sup>	114.25±3.75 <sup>D</sup>	152.40±7.83 <sup>B</sup>	151.76±5.72
No. of Segments	10.10±0.16 <sup>D</sup>	10.75±0.22 <sup>B</sup>	11.20±0.22 <sup>A</sup>	10.40±0.18 <sup>C</sup>	10.61±0.10
Juice (ml)	63.00±2.34 <sup>C</sup>	104.50±2.94 <sup>A</sup>	56.50±1.80 <sup>D</sup>	65.00±1.27 <sup>B</sup>	72.25±3.16

Means having similar letters in a column or in a row are statistically non-significant (P>0.05)

**Table-2: Comparison of means±SE (Days x Variety) for acidity (%)**

Days	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	Days x Variety
0 Day	0.062±0.003J	0.123±0.003G	0.122±0.003IG	0.133±0.003F	0.1099±0.003C
45 Days	0.082±0.003I	0.230±0.002B	0.173±0.003E	0.183±0.002D	0.1670±0.003B
90 Days	0.100±0.005H	0.304±0.007A	0.216±0.006C	0.226±0.005B	0.2105±0.003A
Means	0.080±0.002D	0.220±0.002A	0.170±0.002C	0.181±0.002B	

Means having similar letters in a column or in a row are statistically non-significant (P>0.05). Within box means express interaction means.

**Table-3: Comparison of means±SE (Treatment x Variety) for acidity (%)**

Treat.	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	Treat x Variety
T0	0.088±0.008I	0.247±0.011A	0.243±0.009A	0.251±0.008A	0.207±0.005A
T1	0.072±0.001J	0.206±0.001E	0.132±0.001H	0.147±0.002FG	0.139±0.004D
T2	0.081±0.003IJ	0.222±0.004BC	0.226±0.004B	0.227±0.004B	0.190±0.004B
T3	0.074±0.002SJ	0.209±0.002DE	0.135±0.002H	0.148±0.003FG	0.142±0.004D
T4	0.081±0.002IJ	0.215±0.001C-E	0.141±0.001GH	0.155±0.002F	0.148±0.004C
T5	0.082±0.004IJ	0.217±0.004B-D	0.143±0.004GH	0.157±0.005F	0.150±0.004C

Means having similar letters in a column or in a row are statistically non-significant (P>0.05). Within box means express interaction means.

T<sub>0</sub>= Control (citrus juice), T<sub>1</sub>= preservative juice (0.075% KMS), T<sub>2</sub>= Pasteurization 81 °C for 90 sec, T<sub>3</sub>= preservative (0.075% KMS) & 90 sec Pasteurization 81 °C, T<sub>4</sub>= 04 mints Ultrasound & 90 sec Pasteurization 81 °C, T<sub>5</sub>= 04 mints Ultrasound

**Total phenolic content (TPC) of citrus juices**

Total phenolic contents of Musambi juice showed (Table-5 and Figure-2) prominent increase in thermo-

sonicated and sonicated juice in comparison to non-sonicated juice. The maximum increase 458.89±6.72 µg/mL was observed in T4 and 450.37±9.83 µg/mL of



gallic acid equivalent T5 as compare to control 317.85±25.02 µg/mL of gallic acid equivalent. Similar trend in total phenolic content was observed in juices of other citrus varieties.

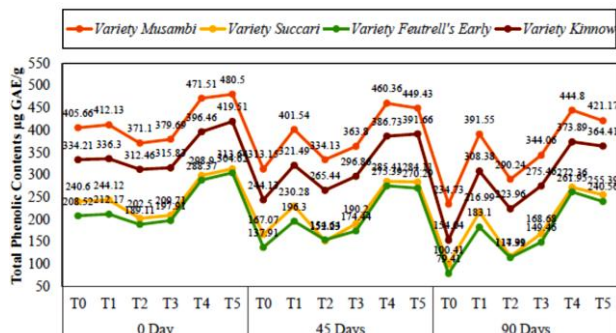


Figure-2. Comparison of means (Treatment x Days x Variety) for total phenolic contents µg GAE/g

Table-4: Comparison of means±SE (Days x Variety) for total phenolic contents (TPC) (µg GAE/g)

Days	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	
0 Day	420.10 ±10.72A	251.58 ±10.37F	233.45 ±11.26G	352.46 ±10.23C	314.40 ±7.43A
45 Days	387.07 ±13.71B	218.22 ±12.97H	201.49 ±13.19I	317.72 ±13.76D	281.12 ±7.81B
90 Days	354.43 ±18.08C	188.52 ±15.98I	171.58 ±15.89J	283.36 ±18.84E	249.47 ±8.55C
Means	387.20 ±8.99A	219.44 ±8.32C	202.17 ±8.44D	317.84 ±9.19B	

Means having similar letters in a column or in a row are statistically non-significant (P>0.05). Within box means express interaction means.

The minimum decrease of total phenolic contents was observed in the juice of Musambi 15.63 % while the maximum reduction was observed in the juice of Feutrell's Early 26.50 % during storage (Table 4). The total phenolic contents of juices of Musambi, Succari, Feutrell's Early and Kinnow significantly decreased during storage of 90 days. The maximum increase in total phenolic content has been observed in juice of Musambi while minimal increase was observed in juice of Feutrell's Early (Table 4). The total phenolic contents gradually decreased during storage. The maximum value of total phenolic content in fresh Musambi juice 420.10±10.72 µg/mL of gallic acid equivalent was found at start of storage. However, it decreased significantly at 90 days of storage 354.43±18.08 µg/mL of gallic acid equivalent. The combined effect of pasteurization and sonication

shows better retention of total phenolic contents in juices of Musambi, Succari, Feutrell's Early and Kinnow as well as during the storage period of 90 days as compare to that of which were preserved chemically, pasteurized or sonicated alone.

Phenolic components are the essential components of fruits due to their powerful antioxidant behavior. Phenolic components in fruits plays a vital role in organoleptic properties such as color, flavor and bitterness. That's why phenolics are considered as the quality parameter for fruits and its products (Kreutzmann et al., 2008; Zhang et al., 2005; Nacz and Shahidi, 2003).

Table-5: Comparison of means±SE (Treatment x Variety) for total phenolic contents (TPC) (µg GAE/g)

Treat.	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	
T0	317.85 ±25.02D	169.36 ±20.42IJ	141.95± 18.78K	244.13± 26.23G	218.32± 11.23E
T1	401.74 ±5.50B	230.46 ±5.08G	197.19± 4.68H	322.06± 5.32D	287.86± 9.39B
T2	331.82 ±12.06D	157.35 ±12.59JK	152.91± 11.26JK	267.28± 13.22F	227.34± 9.43D
T3	362.51 ±6.64C	189.53±6.40HI	173.94± 7.47IJ	296.05± 6.59E	255.51± 8.99C
T4	458.89 ±6.72A	285.56±4.86E F	275.24± 5.64EF	385.69± 4.67B	351.34± 9.65A
T5	450.37 ±9.83A	284.38±9.58E F	271.82± 10.39F	391.86± 8.84B	349.61± 9.94A

Means having similar letters in a column or in a row are statistically non-significant (P>0.05). Within box means express interaction means.

T<sub>0</sub>= Control (citrus juice), T<sub>1</sub>= preservative juice (0.075% KMS), T<sub>2</sub>= Pasteurization 81 °C for 90 sec, T<sub>3</sub>= preservative (0.075% KMS) & 90 sec Pasteurization 81 °C, T<sub>4</sub>= 04 mints Ultrasound & 90 sec Pasteurization 81 °C, T<sub>5</sub>= 04 mints Ultrasound

Nadeem et al. (2018) checked that effect of ultrasound and chemical treatment on total phenolic content on carrot-grape juice blend during storage. They found that sonicated juice blend showed significant rise in the total phenolic contents.

Kaur and Aggarwal (2015) checked the effect of thermal processing and chemical preservation on storage stability of tomato juice. They found total phenolic content 47.39-47.87 mg/100g respectively. The chemically preserved juice showed the better total phenolic content retention than thermally treated samples. The decrease was observed in storage period



of 3, 6 and 9 months of total polyphenol content of tomato juices.

The results of this study were in favor of research conducted on kasturi lime juice (Bhat et al., 2011). The significant increase in the total phenolic contents thought to be due the production of cavitation by the distraction of cell wall that causes the release of some chemically bound total phenolic contents and eventually increase their availability in the juice. The bubbles are formed due to the creation of hydroxal radicals by the implementation of sonication treatment, increased the availability of total phenolic content in citrus juice. Enhancement in the activity of polyphenolic oxidase might be the reason for increase in total phenolic contents.

### Total antioxidant activity of citrus juices

Antioxidant activity of Kinnow showed (Table-7 and Figure-3) significant difference in thermo-sonicated and sonicated juice in comparison to non-sonicated juice. The increase was maximum in T4 1,103.20±9.94 µg/mL and T5 1,110.74±23.67 µg/mL respectively in comparison to control 929.61±38.10 µg/mL of ascorbic acid equivalent. Similar trend in antioxidant activity was observed in juices of other citrus varieties (Table-7).

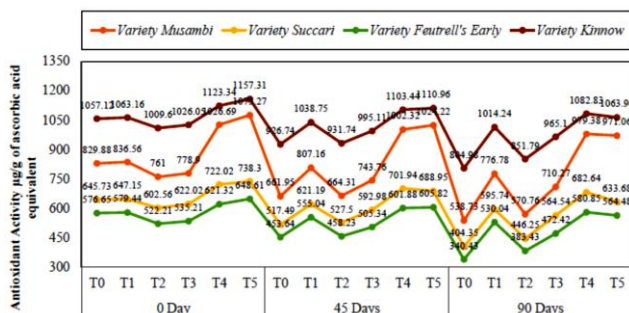


Figure-3. Comparison of means (Treatment x Days x Variety) for total antioxidant activity (TAA)

The minimum decrease of antioxidant activity 10.16 % was observed in the juice of Kinnow while the maximum reduction 17.56 % was observed in the juice of Feutrell's Early during storage (Table-6). The antioxidant activity of juices of Musambi, Succari, Feutrell's Early and Kinnow significantly decreased during storage of 90 days. The maximum overall rise in antioxidant activity has been found in juice of kinnow while minimal increase was observed in juice of Feutrell's Early (Table-6). The antioxidant activity gradually decreased with the passage of time. The maximum value of antioxidant activity in fresh

Kinnow juice 1,072.76±15.40 µg/mL of ascorbic acid equivalent was found at start of storage. However, it decreased significantly 963.81±26.89 µg/mL of ascorbic acid equivalent at 90 days of storage. The combined effect of pasteurization and sonication shows better retention of antioxidant activity in juices of Musambi, Succari, Feutrell's Early and Kinnow as well as during the storage period of 90 days as compare to that of which are preserved chemically, pasteurized or sonicated alone.

Ultra-sonication disrupts the bound antioxidants for example ascorbic acid and phenolic contents which lead to the increased total antioxidant activity. Additionally, ultrasonic technique inactivates enzymes, for instance, polyphenol oxidases which are responsible for enzymatic browning leading to enhanced total antioxidant activity values. The samples which have high number of polyphenolic compounds has more antioxidant capacity. Sonication plays significant role in enhancing the total antioxidant activity of citrus juice by increasing its phenolics.

Nadeem et al. (2018) checked that effect of ultrasound and chemical treatment on antioxidant properties on carrot-grape juice blend during storage. They found the significant difference in total antioxidant activity, as sonicated samples show more antioxidant activity as compare to non-sonicated samples. During storage the prominent difference were observed. From day 1 to 90, the total antioxidant activity decreased of all treated samples significantly. The highest antioxidant was observed in 6 min ultrasonication treatment 792 mg/100 mL Juice while lowest antioxidant was observed in control 511 mg equivalent of Trolox/100 mL Juice.

Kaur and Aggarwal (2015) described that the antioxidants inhibits the formation of polymerization chain which might be occur due to the free radicals and other subsequent oxidizing reactions which ultimately reduces the oxidation process. According to their results, percent antioxidant activity for samples on the day of preparation to 06 months were 45.77 to 41.92 percent respectively. They found the non-significant (p≤0.05) decrease in antioxidant activity during storage period of 6 months.

### Total plate count of citrus juices

Total plate count of Feutrell's Early juice showed (Table-9 and Figure-4) significant reduction in thermo-sonicated (T4) and in combination chemically preserved and pasteurized (T3) juice in comparison to sonicated (T5) and non-sonicated (T1 and T2) juice.

This decrease was maximum in T3  $1.56 \pm 0.010 \log_{10}$  cfu/mL and T4  $1.66 \pm 0.019 \log_{10}$  cfu/ mL respectively as compared to control  $2.16 \pm 0.095 \log_{10}$  cfu/ mL, chemically preserved  $1.84 \pm 0.040 \log_{10}$  cfu/ mL and pasteurized  $1.78 \pm 0.03 \log_{10}$  cfu/ mL. Similar trend in total plate count was observed in juices of other citrus varieties. The maximum overall decrease in total plate count has been found in juice of Musambi while minimal decrease was observed in juice of Feutrell's Early (Table-8).

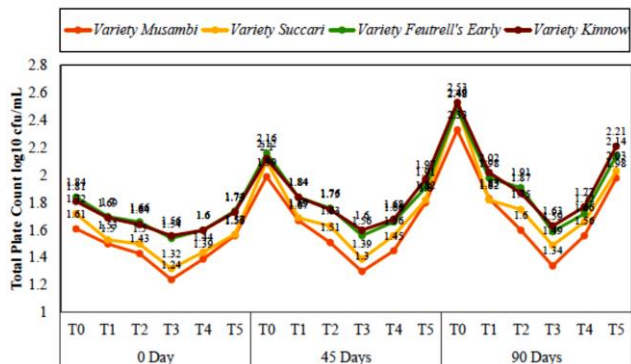


Figure-4. Comparison of means (Treatment x Days x Variety) for total plate count cfu/mL

The total plate count of juices of Musambi, Succari, Feutrell's Early and Kinnow significantly increased during storage of 90 days. Total plate count gradually increased during storage. The maximum value of total plate count in fresh Musambi juice  $1.46 \pm 0.030 \log_{10}$  cfu/ml was found at start of storage. However, it

increased significantly  $1.77 \pm 0.079 \log_{10}$  cfu/ml at 90 days of storage. The combined effect of pasteurization and chemical preservation and also thermos-sonication shows better reduction of total plate count in juices of Musambi, Succari, Feutrell's Early and Kinnow as well as during the storage period of 90 days as compare to control or sonicated samples alone. The better reduction in total plate count might be due to the implementation of combined treatment of heat and ultrasonication as some microbes are sensitive to heat and killed by the heat treatment.

Zou and Jiang (2016) sonicated carrot juice for 20, 40 and 60 minutes and examined the microbial load in comparison to non-sonicated juice sample. They have observed significant decrease in total plate count of sonicated carrot juice as compared to blank. The maximum reduction in total plate count of carrot juice was noticed in 60 minutes sonicated juice as compared to control.

Abid et al. (2014) studied the thermo-sonication as a potential quality enhancement technique of apple juice. They have observed the impact of thermo-sonication on total plate count. They illustrated that the total plate count is highly sensitive to heat. They have achieved the complete inactivation of microbes at 60 °C as compare to sonication method and found the significant reduction in total plate count. Their results showed that the combined effect of ultrasonication and heat reduced the adverse changes in the food and also has the increased level of microbial inactivation of apple juice.

Table-6: Comparison of means±SE (Days x Variety) for antioxidant activity (AA) (µg ascorbic acid/g)

Days	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	Days x Variety
0 Day	884.72±31.15D	662.96±13.54G	580.57±11.89HI	1,072.76±15.40A	800.25±16.46A
45 Days	817.29±37.22GE	608.34±18.32H	529.99±15.92J	1,017.79±19.97B	743.35±16.84B
90 Days	757.83±43.36F	554.53±24.86I	478.61±22.32K	963.81±26.89C	688.70±17.68C
Means	819.95±22.44B	608.61±12.59C	529.73±11.32D	1,018.12±13.51A	

Means having similar letters in a column or in a row are statistically non-significant (P>0.05). Within box means express interaction means.

Table-7: Comparison of means±SE (Treatment x Variety) for antioxidant activity (AA) (µg ascorbic acid/g)

Treat.	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	Treat x Variety
T0	676.86±43.89F-H	522.52±35.36L-N	456.91±34.65MN	929.61±38.10D	646.47±23.89D
T1	806.83±14.46E	621.36±12.78H-J	554.84±10.96J-L	1,038.71±14.09BC	755.44±21.67B
T2	665.36±29.00G-I	525.44±24.67K-M	454.63±21.32N	931.04±26.39D	644.12±22.59D
T3	744.31±11.41EF	593.18±10.18JK	504.32±11.68L-N	995.42±15.24CD	709.31±21.86C
T4	1,002.80±24.06C	702.20±9.38FG	601.35±8.96IJ	1,103.20±9.94AB	852.39±23.83A
T5	1,023.51±26.25C	686.98±19.61F-H	606.31±14.14IJ	1,110.74±23.67A	856.89±25.19A

Means having similar letters in a column or in a row are statistically non-significant (P>0.05). Within box means



express interaction means.

$T_0$ = Control (citrus juice),  $T_1$ = preservative juice (0.075% KMS),  $T_2$ = Pasteurization 81 °C for 90 sec,  $T_3$ = preservative (0.075% KMS) & 90 sec Pasteurization 81 °C,  $T_4$ = 04 mints Ultrasound & 90 sec Pasteurization 81 °C,  $T_5$ = 04 mints Ultrasound

**Table-8: Comparison of means±SE (Days x Variety) for total plate count (T-P-C) (log<sub>10</sub> cfu/ mL)**

Days	Variety				Means
	Musambi	Succari	Feutrell's Early	Kinnow	Days x Variety
0 Day	1.46±0.030H	1.52±0.030G	1.68±0.024E	1.67±0.020E	1.58±0.011C
45 Days	1.62±0.056F	1.70±0.054E	1.82±0.047C	1.83±0.044C	1.74±0.018B
90 Days	1.77±0.079D	1.87±0.078B	1.97±0.071A	2.01±0.073A	1.91±0.025A
Means	1.62±0.038D	1.69±0.038C	1.82±0.033B	1.84±0.034A	

Means having similar letters in a column or in a row are statistically non-significant ( $P>0.05$ ). Within box means express interaction means.

## Conclusion

Thermo-sonication elicited better retention of total phenolic contents and total antioxidant activity without any significant impact on titratable acidity of citrus juices in comparison to the pasteurized and chemically preserved citrus juices. Additionally, thermo-sonication showed significant reduction in total plate count. These results proposed that thermo-sonication technique may effectively be used for the processing of citrus juices with improved functional properties.

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### Contribution of Authors

Shahid MB & Nadeem M: Conceptualized the study, designed research methodology, analysed data and wrote original draft and approved final draft

Murtaza MA: Helped in laboratory analysis, reviewed literature and approved the final draft

Rukh L: Data analysis and interpretation

Shaukat A: Performed laboratory analysis and reviewed literature

Riaz MN: Contributed in article write up and editing

