Synergistic Effects of Nisin-Assisted Thermosonication on Quality Characteristics of Apple Juice

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Abstract
Awareness in consumers towards the safety and quality of food is increasing day by day, that is why the demand for safe and nutritious food has been increased. The present study was conducted to evaluate the synergistic effects of bio preservative nisin (NS) with thermosonication (TS) on the quality and safety attributes of apple juice. The purpose of conducting the given research was to check the effects of chemically assisted TS and to provide microbial safety to the apple juice without thermal degradation. Treatments were applied by using TS (24kHz, 30^{o}C, 50min) in T_1 and at 60^{o}C in T_2, after that NS was added in treated samples at the concentration of 75 mg/L in T_3 and 100 mg/L in T_4, samples were analyzed with the interval of 5 days during storage of 15 days. Final results, stated that value of acidity and cloud value increased from (0.34±0.04) to (0.41±0.09) and (0.09 ± 0.02) to (1.13 ± 0.05) respectively after applying the treatment of TS and TS+NS, but decreasing trend were seen during storage interval. Treatments increased the total phenolic contents of juice from (274.53) to (291.60) in T_4 followed by T_3 but decrease to (288.45) after storage interval of 15 days. On the other hand, the significant decrease was seen during treatment and storage in the pH from (3.93) to (3.88) in T_4 and ascorbic acid from (4.09) to (3.68 mg/100mg) due to heating and increase in storage time. Nonsignificant results showed in the TSS during storage and after treatments. Treatments like TS and TS+NS improved the color of apple juice, which gave decreasing trend during storage. Total plate count decreased significantly after the treatments like T_4 (TS+NS) and T_3 from 1.97 log to 1.53 log, but microbial load increased a little bit during storage; efficiently retained by T_4 treatment. All the treatments improved in the overall acceptability of apple juice. Therefore, the findings of the given study analyzed that TS+NS treatment (T_4 & T_3) are better methods for providing quality retention and microbial safety of apple juice without thermal degradation as compared to simple TS treatment (T_1 & T_2), but both TS and TS+NS have the potential to retain quality in apple juice. Hence; the study concluded that TS+NS is an effective method to decrease microbial load without thermal degradation of quality characteristics.

Keywords: - Thermosonication, Nisin, Apple Juice

Introduction

Fruits and vegetables contain antioxidants and bioactive compounds that are required for the normal functioning and development of the human body. Fruits contains large number of antioxidants, vitamins, and minerals but low in fats. Among fruits apple (*Malus domestica*) is considered one of the most valuable fruit, with global production of 85 million tones and a market value of 45 billion dollars in 2019. Apple is generally known as the "sweet gold. Pakistan has a strong orchard legacy, particularly around harvest season. According to given data Pakistan is on 25th number for apple production, on world level its production is 556000 tons/year (GOP, 2017-18). Phytochemicals are present in most of the fruits. Wide range of functions are attached with these phytochemicals (Perez *et* al., 2010). These help in the prevention

of chronic disease for example, defend against lipid oxidation and are engaged in processes that lead to the formation of additional oxidants. When it came to antioxidant capabilities, apples came in second place. When compared to other fruits, apples placed second in terms of antioxidant qualities.

Apple juice contains a lot of high-value compounds. Flavonoids and phenolic acid are examples of high antioxidant components. The antioxidant levels vary depending on the type of fruit, climate, and gardening conditions. Includes other fruit parts such as pulp and skin the peel section has a lot of sugar. These components have higher antioxidant action than phenolic components. These components play an important role in protecting our bodies from disorders like immune system degradation, oxidative stress causes asthma, cardiac disease, and diabetes (Li *et al.*, 2021). Apple juice is a widely consumed beverage around the world due to the nutritional qualities and distinct flavor attributes. When determining the quality and features of apple juice, the aroma is an important factor to consider. Apple juice contains sugar 39%, protein 0.37%, potassium 7% very low fat 2% a variety of volatile components, including esters, ketones, fatty acids, alcohols, aldehydes, and terpenoids, among others also contains vitamin C, flavonoids and phenolic contents with an amount of total 11% (Aguilar *et al.*, 2017).

Many nutrients present in fruit juices are depleted by the use of high heat in traditional preservation techniques like antioxidants are depleted in fruit juices when they are processed traditionally. Lycopene is lost due to oxidation and isomerization during thermal processing. Loss of vitamin C occurs due to processing with the high-temperature and vitamin C high water content renders it susceptible to deterioration (Putnik *et al.*, 2020). So, we should apply some non-thermal techniques to reduce these damages in the fruit juices. Non-thermal food processing like sonication and thermosonication provide microbial safety to the food and improve the quality without thermal degradation (Aadil *et al*., 2017).

Microbes are a big source of damage in fruit juices. Microbes damage the quality of juices as well as cause different diseases in the consumers (pathogens). So, reduction in these microbes is the necessity of time to provide microbial safety to fruit juices and this can be done by synergistic application of some chemicals (bio preservatives) with TS. For these purposes nisin seems to be the best chemical to apply in food products (Wang *et al.*, 2018). *Lactococcus lactis* spp. *lactis* produces nisin, a bacteriocin which widely effects the gram-positive bacteria and gram-negative bacteria as well. In more than 55 countries, nisin is an approved preservative in a variety of foods.

Nisin is the only approved peptide of this type has found broad use in food processing. Nisin is a bacteriocin that has a high inhibitory impact on most gram-positive bacterial spores, but it has low effect on gram-negative bacteria, fungi, or viruses. Nisin can be used as a bio preservative in fruit juices with the standard amount range of 1-25 ppm. The results revealed that eliminating wide range of gram-positive and gram-negative like *Alicyclobacillus acidoterrestris* and *Alicyclobacillus* contaminants spores in kiwi fruit juice with the bacteriocin nisin was successful. The factors which effect the activity of nisin are concentration, temperature and duration of holding (Ekhtelat *et al.*, 2020).

The addition of nisin causes the significant decrease in *E. coli* in the apple juice. The findings suggest that nisin with high-pressure technique is a viable method for inactivation of many pathogen juices of fruits and vegetables (Soto *et al.*, 2019). In apple juice both nisin and lysozyme may be utilized to stop *Alicyclobacillus acidoterrestris* cells growth and it is greatly affected by storage duration (Molva and Bayasal, 2017).

Objectives:

Based on the need of project research study objectives include:

- To evaluate the effects of nisin assisted thermosonication on the quality attributes of apple juice
- To provide the microbial safety of the apple juice without thermal degradation

Methodology

Apparatus and chemical used

Chemicals

Sodium hydroxide (NaOH) Aluminum chloride (AlCl3)
Sodium nitrite (NaNO2)
Sodium carbonate (Na2CO3)
2,2, -diphneyl-1-picrylhydrazyl radical (DPPH)
B-Carotene
Folin-Ciocaltcu reagent
Catechin
Ascorbic acid
Acetone
n-Hexane
Methanol
Gallic acid

Procurement of Apples

Fresh, perfectly matured apples were purchased from the Faisalabad local market. The high-quality fruits were taken to a laboratory for further processing.

Procurement of Nisin

The required amount of bio preservative nisin was brought from the Chemical Center, Jinnah Colony, Faisalabad.

Grading and sorting

To improve the efficiency of the research, apples were graded and sorted based on their size, color, and precise quality.

Sample preparation

Apples were peeled using a clean stainless-steel knife, and the seeds were removed from the inside. After that, the blender was used for extraction. The freshly obtained apple juice was then run through the finisher again to remove any remaining seeds or pulp. The freshly extracted juice was then taken for further analysis. Following that, the apple juice was divided into four equal samples: T_0, T_1, T_2, T_3 and T_4. T_0 was used as the control sample. T_1 was treated with TS, T_2 with variable temperature TS, T_3 and T_4 with both TS+NS treatment.

Procedure

Thermosonication treatment

Thermosonication treatment was applied on the sample of 350ml with the help of thermorasonic transducer fixed at the frequency range of 24 kHz present in Bio Safety Lab. NIFSAT, UAF with constant temperature of 30°C in T_1 and 60°C in T_2 for 50 minutes respectively, and treated juice samples were stored at 4°C for 15 days after each treatment.

After the treatment of samples T_1 and T_2 with TS, nisin was applied at the concentration of 75mg/L to 100 mg/L respectively in T_3 and T_4, the treated juice sample was stored at 4°C for 15 days after each treatment as like Molva and Bayasal (2017).

Treatment Plan

Treatments	Sample	Application
T_0	Control	No treatment applied
T_1	Apple	TS (24 kHz, 30°C) 50min
T_2	Apple	TS (24 kHz, 60°C) 50min
T_3	Apple	TS+NS (24 kHz+75 mg/L, 30°C) 50 min
T_4	Apple	TS+NS (24 kHz+100 mg/L, 60°C) 50 min

Physiochemical analysis

Physiochemical analysis was performed to check the effects of nisin assisted thermosonication and storage interval on the pH, vitamin C, titratable acidity, cloud value, and TSS (°Brix).

Titratable acidity (TA)

Principle

The standard alkali (0.1 N NaOH) is used to titrate against a measured amount of the sample in the presence of an indicator or dye which is phenolphthalein until the appearance of light pink color. The titratable acidity is expressed in percentage. The factor for the calculation of titratable acidity is taken based on the predominant acid concentration in the product that is being analyzed for acidity.

Procedure

Titratable acidity of the fresh apple juice sample was determined according to (Dars *et al.*, 2019). The titrimetric method was used to standardize the acid present in the developed juice with the help of standard alkali 0.1 N NaOH and phenolphthalein. 10 milliliter of the sample was taken in the graduated beaker. 250 milliliter of distilled water was taken in the graduated beaker of 500ml capacity. 2-3 drops of phenolphthalein dye were added to the solution. The 0.1 N solution of NaOH after preparation (dissolving 4 g of NaOH in 1 liter of distilled water) was taken in the titration tube and titrated against acid present in the beverage. The used volume of 0.1N NaOH was measured until the light pink color appeared in the beaker. Note down the original volume of alkali and the final volume after titration. As the predominant acid present in the beverage was malic acid, so, the equivalent weight of malic acid was used for the calculation factor, and average of acid was used in calculation factor. Titratable acidity is expressed as a percentage.

Titratable acidity % = 0.1 × acid factor × Normality of the NaOH × Volume of NaOH used for titration×100/volume of sample in ml.

pH

The pH of fresh apple juice was determined according to the AOAC (2019). The pH meter was standardized using buffer solutions having pH 4 and 7. A well representative sample individually from all the treatments was taken in a graduated glass cylinder. After that knob of the pH meter was washed with the help of distilled water and then immersed in the sample, after some time when varying values on the pH meter stop then that value was recorded. For each new sample/treatment first the pH meter was standardized, the knob was washed with the help of distilled water and then the pH of the sample was performed.

Total soluble Solids (TSS)

Total soluble solids state the whole number of soluble solids present in the product or the sweetness of the liquid. The refractometer is the instrument used to measure the total soluble solids as degree Brix. Refractometers work on the basis that as a substance's density rises (for example, when sugar is dissolved in water), so does its refractive index. A prism with a substantially higher refractive index than the sample solution to be tested is used in refractometers. The Prism of the refractometer was cleaned with distilled water and the value was adjusted to zero then some drops of the juice sample

were dropped on the clear prism of the refractometer so that the prism is thoroughly covered with the liquid then the value was recorded after visualizing from the lens. For each new sample the prism was first cleaned with the help of distilled water or tissue paper and adjusted to zero then drops of the next sample were dropped onto the prism and in this way, the next value of another sample was recorded.

Ascorbic acid (vitamin C)

Ascorbic acid of apple juice was determined by using (2, 6-dichlorophenol indophenol) dye, according to the method of AOAC (2019).

Principle

The indicator dye is reduced to a colorless solution by ascorbic acid. 2,6-dichlorophenol indophenol is rendered colorless by ascorbic acid. Ascorbic acid has a unique reaction at pH 1-3.5, which is only possible at this pH range. In acidic solutions, the indicator dye turns red, whereas in alkaline solutions, it turns blue. After filtering with (Whatman® No# 1 Filter paper), fruit juice was extracted from all samples. The quantity of ascorbic acid in the food sample is determined by the volume of titration used.

Preparation and standardization of dye

Soda benzoate, sodium dichlorophenol indophenol and the distilled water were measured out, dissolved in the final volume of 250ml, and mixed well. To produce another Oxalic acid solution, 1 liter of distilled water was mixed with 4 grams of oxalic acid salt. Using 2ml of that ascorbic acid solution, an indicator dye solution was titrated against it until the appearance of a pink color lasting 15-20 seconds was attained.

Titration

A 30 ml sample from each treatment was individually mixed with 70ml of 0.4% oxalic acid solution. All these contents were transferred to a 100ml volumetric flask. 15ml of diluted beverage sample was collected after filtration using filter paper in another flask and titrated against indicator dye until the appearance of the pink color and that color persisted for about 15 seconds. Three consecutive values were taken for each sample.

Calculation

Vitamin C contents in the sample were calculated according to the given formula,

Ascorbic Acid (mg/100g) = (D1× V) / (D × A × B) × 100

Where;

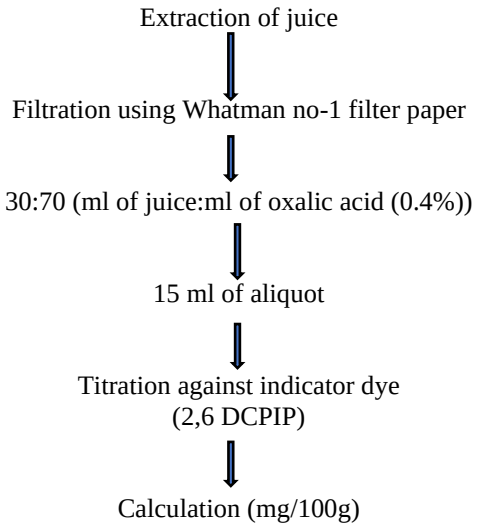
D1= milliliter of the indicator dye used in the titration of aliquot

V= Aliquot volume prepared by adding 0.4% of oxalic acid solution

B= milliliter of aliquot used for the titration process

A= milliliter of juice used

D= Dye (ml) used in the titration procedure of solution of standard ascorbic acid (1-ml) formulated by the addition of 0.1% of ascorbic acid (1-ml) + 0.4% of oxalic acid (1.5ml)



Cloud value

Cloud value in fruit juices is related to the particle suspensions and it is a desirable quality parameter in apple juice. The cloud value of an apple juice sample was measured using Ertugay and Baslar (2014) method with some slight modifications. A 5ml juice sample was centrifuged at 3000rpm for 10 minutes, with the temperature kept at 20°C

throughout. Cloud value was determined using a spectrophotometer using pure water as a blank. At 660 nm, the absorbance of the supernatant was measured.

Total phenolic contents analysis

Total phenolic contents were founded by using UV Spectrophotometers by using Follin-Ciocalteu method explain by Sharif *et al*. (2018). In this process Follin-Ciocalteu reagent was used. Took 1 ml of juice sample and added 1 ml aliquot of 0.10mg/ml gallic acid solution in methanol was mixed with 5ml of Follin-Ciocalteu reagent (diluted by 10 times with distilled water) and added 4 ml of Na_2CO_3 (20%). Then this sample was examined under UV spectrophotometer at the resonance of 765nm for 1 hour. Total phenolic contents were calculated in gallic acid equivalent GAE mg/100ml (Pandey *et al.*, 2018).

Microbial analysis

Total plate count is the method used to measure the total amount of microorganisms present in the sample. It measures the total viable microbes present in the sample. Nutrient agar is used as a growth medium for total plate count. Growth media was prepared according to the standard protocol dissolving 14 grams of agar in 500 milliliters of distilled water with continuous mixing and heating on a hot plate for a while until the clear color appears and all the agar was properly dissolved in the distilled water. After that, the prepared media is tightly covered with aluminum foil. Similarly, peptone water was prepared according to the standard method after mixing peptone salt in the required amount of distilled water and then putting that graduated flask on a hot plate for some time until the color changed and peptone salt properly dissolved in the water.

All the required test tubes according to the different treatments and dilutions were first washed and dried then each test tube was filled with 9 ml of peptone water and covered with aluminum foil. After that, all the test tubes filled with peptone water and nutrient media were kept in the autoclave for a period time of 15 minutes until the autoclave attains a temperature of 121^{o}C. After the autoclave had been done and the temperature of the autoclave reaches below 80^{o}C, then the lid of the autoclave was opened so that the entrapped steam in the autoclave moves out. After the autoclave was done all the required material was placed in laminar airflow and growth media was poured onto the surface of the required petri dishes. After those different dilutions were performed according to the required test tubes that were already marked according to treatment and dilution number. 1ml from the sample was poured into the required test tube and a series of dilutions was performed after taking 1ml of solution from that test tube and pouring it into the next test tube. 1ml of the solution was taken from the test tube and poured into the petri dish with culture media on it. After that, all the petri plates were wrapped with the help of pipette tape and kept in the incubator for 24 hours. The next day colonies were counted on the colony counter. The readings then converted into logs CFU/ml by calculating the colonies into Log^{10}.

Total plate count log CFU/ml = Log^{10} (Number of colonies)

Color analysis

Treated juice samples were analyzed on the color parameters including L*(indicate lightness-darkness), a*(identify redness-greenness), b* (indicate yellow-blue) values by using colorimeter Farias *et al*. (2020). 2 to 3ml of every sample were poured in to the petri dishes with shaking it gently to spread the sample thoroughly on the surface of petri dish. Then these samples were examined under the digital colorimeter and reading were taken after capturing.

Sensory evaluation

The sensory evaluation of apple juices was conducted by a trained panel of ten assessors. The panelists were trained about the hedonic scale and the properties of juice under concentration and asked to rate the apple juices based on their odor, taste, flavor and overall acceptability characteristics. Using the Wlodarska *et al*. (2016) approach, apple juice samples were sensory analyzed and graded on a 9-point hedonic scale. Instructed to fill out a nine-point hedonic scale sensory sheet, the panelists were asked to record their impressions (9 points for extremely like and 1point for extremely dislike).

Statistical analysis

Collected data will be tested through standard statistical analyses of juice to determined symmetrical means and level of significance according to the procedure of Montgomery, (2017). The data were expressed as mean ± standard deviation (SD). The SPSS 8.1 (SPSS, Chicago, USA) was utilized of Tukey's test and two-way analysis of variance for multiple comparisons. The statistically analysis was considered significant difference if $P < 0.05$.

Results and Discussion

Mean values observed for titratable acidity %

Treatment	**Storage (Days)**			
	0	5	10	15
T_0	0.34 ± 0.04^{e}	0.36 ± 0.08^{e}	0.45 ± 0.06^{c}	0.51 ± 0.05^{a}
T_1	0.38 ± 0.06^{c}	0.39 ± 0.07^{bc}	0.46±0.09b	0.48 ± 0.07^{c}
T_2	0.43 ± 0.09^{a}	0.45 ± 0.06^{a}	0.47 ± 0.04^{a}	0.49 ± 0.09^{b}
T_3	0.37 ± 0.07^{d}	0.39 ± 0.05^{d}	0.43 ± 0.07^{d}	0.44 ± 0.06^{e}
T_4	0.41 ± 0.09^{b}	0.43 ± 0.04^{b}	0.44 ± 0.05^{e}	0.45 ± 0.07^{d}

Mean values observed for pH

Treatment	Storage (Days)			
	0	**5**	**10**	**15**
T_0	3.91±0.07^{a}	3.87±0.05^{a}	3.81±0.04^{a}	3.71±0.09^{a}
T_1	3.88±0.09^{b}	3.84±0.08^{b}	3.79±0.07^{b}	3.70±0.08^{bc}
T_2	3.84±0.04^{d}	3.80±0.07^{d}	3.75±0.05^{d}	3.67±0.08^{d}
T_3	3.87±0.05^{c}	3.83±0.06^{c}	3.78±0.06^{c}	3.70±0.07^{c}
T_4	3.83±0.04^{e}	3.79±0.05^{e}	3.74±0.03^{e}	3.66±0.05^{e}

Mean values observed for TSS (Brix)

Treatment	Storage (Days)			
	0	**5**	**10**	**15**
T_0	13.03±0.08^{e}	13.08±0.09^{e}	13.16±0.12^{e}	13.29±0.11^{e}
T_1	13.13±0.14^{d}	13.18±0.11^{d}	13.26±0.10^{d}	13.39±0.13^{d}
T_2	13.23±0.09^{b}	13.29±0.07^{b}	13.35±0.12^{b}	13.50±0.13^{b}
T_3	13.17±0.13^{c}	13.24±0.09^{c}	13.31±0.11^{c}	13.42±0.09^{c}
T_4	13.29±0.08^{a}	13.37±0.07^{a}	13.50±0.09^{a}	13.52±0.12^{a}

Mean values observed for ascorbic acid (mg/ 100g)

Treatment	Storage (Days)			
	0	**5**	**10**	**15**
T_0	4.09±0.05^{a}	4.03±0.07^{a}	3.96±0.10^{a}	3.87±0.11^{a}
T_1	3.89±0.07^{c}	3.84±0.06^{c}	3.77±0.09^{c}	3.64±0.11^{c}
T_2	3.67±0.08^{e}	3.63±0.08^{e}	3.56±0.05^{e}	3.47±0.07^{e}
T_3	3.90±0.07^{b}	3.85±0.04^{b}	3.78±0.07^{b}	3.66±0.06^{b}
T_4	3.68±0.04^{d}	3.64±0.05^{d}	3.57±0.06^{d}	3.48±0.05^{d}

Mean values observed for total phenolic contents (mg GAE/ 100g)

Treatment	Storage (Days)			
	0	**5**	**10**	**15**
T_0	274.51±1.56^{e}	268.32±1.43^{e}	261.32±1.39^{e}	253.02±1.56^{e}
T_1	284.82±0.47^{d}	279.53±0.38^{d}	274.36±0.75^{d}	266.86±0.47^{d}
T_2	291.24±0.40^{b}	288.24±0.53^{b}	283.46±0.21^{b}	278.23±0.40^{b}
T_3	285.06±0.46^{c}	281.21±0.24^{c}	278.15±0.62^{c}	275.01±0.46^{c}
T_4	291.64±0.49^{a}	289.78±0.52^{a}	285.46±0.64^{a}	288.62±0.39^{a}

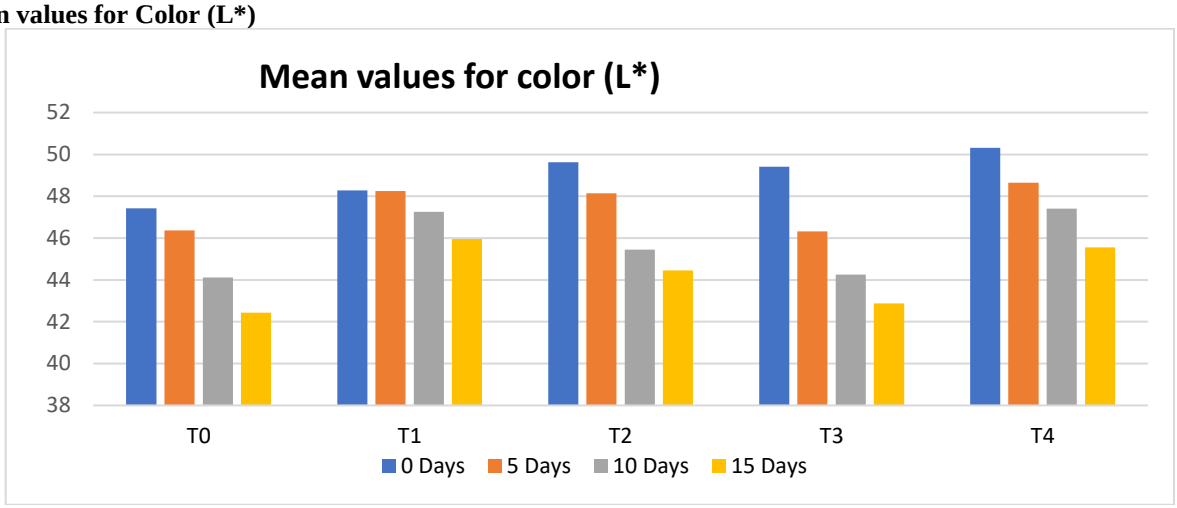
Mean values for cloud value

Treatment	Storage (Days)			
	0	**5**	**10**	**15**
T_0	0.09 ± 0.02^{e}	0.09 ± 0.02^{e}	0.08 ± 0.02^{e}	0.06 ± 0.01^{e}
T_1	0.84 ± 0.06^{d}	0.83 ± 0.08^{c}	0.80 ± 0.07^{d}	0.78 ± 0.09^{d}
T_2	1.03 ± 0.05^{b}	0.97 ± 0.04^{b}	0.95 ± 0.05^{b}	0.92 ± 0.08^{b}
T_3	0.85 ± 0.06^{c}	0.84 ± 0.05^{d}	0.82 ± 0.06^{c}	0.80 ± 0.09^{c}
T_4	1.13 ± 0.05^{a}	1.08 ± 0.06^{a}	1.01 ± 0.08^{a}	0.99 ± 0.11^{a}

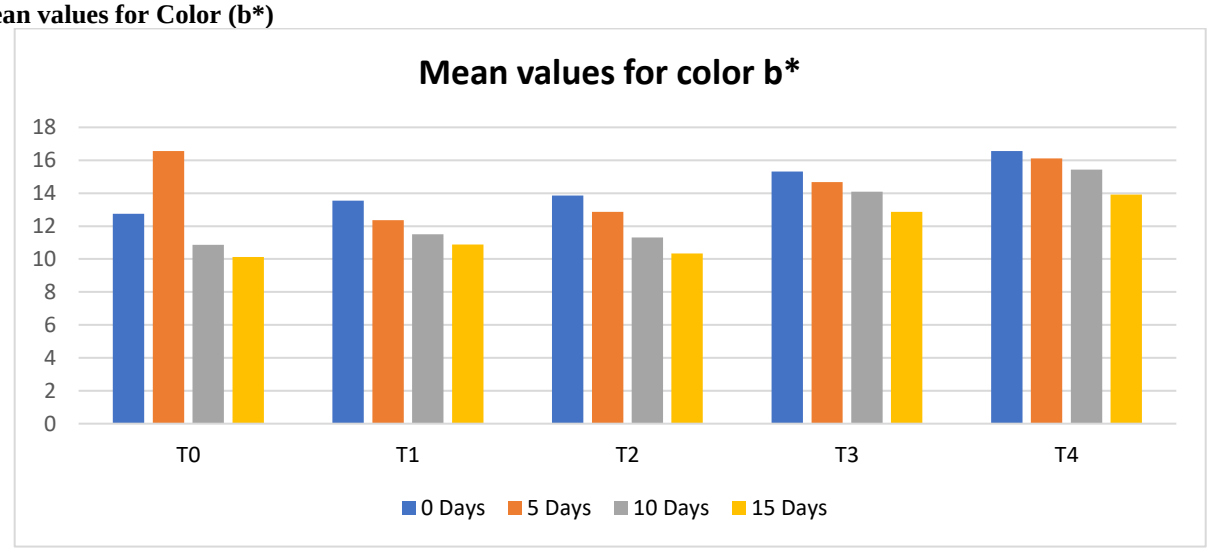
Mean values observed for total plate count (log)

Treatment	Storage (Days)			
	0	**5**	**10**	**15**
T_0	1.97 ± 0.04^{a}	2.0 ± 0.04^{a}	2.09 ± 0.05^{a}	2.20 ± 0.07^{a}
T_1	1.90 ± 0.04^{b}	1.93 ± 0.03^{b}	1.97 ± 0.05^{b}	2.02 ± 0.05^{b}
T_2	1.87 ± 0.03^{c}	1.90 ± 0.04^{c}	1.94 ± 0.03^{c}	1.98 ± 0.06^{c}
T_3	1.71 ± 0.03^{d}	1.74 ± 0.03^{d}	1.78 ± 0.04^{d}	1.86 ± 0.06^{d}
T_4	1.53 ± 0.03^{e}	1.56 ± 0.02^{e}	1.61 ± 0.03^{e}	1.66 ± 0.04^{e}

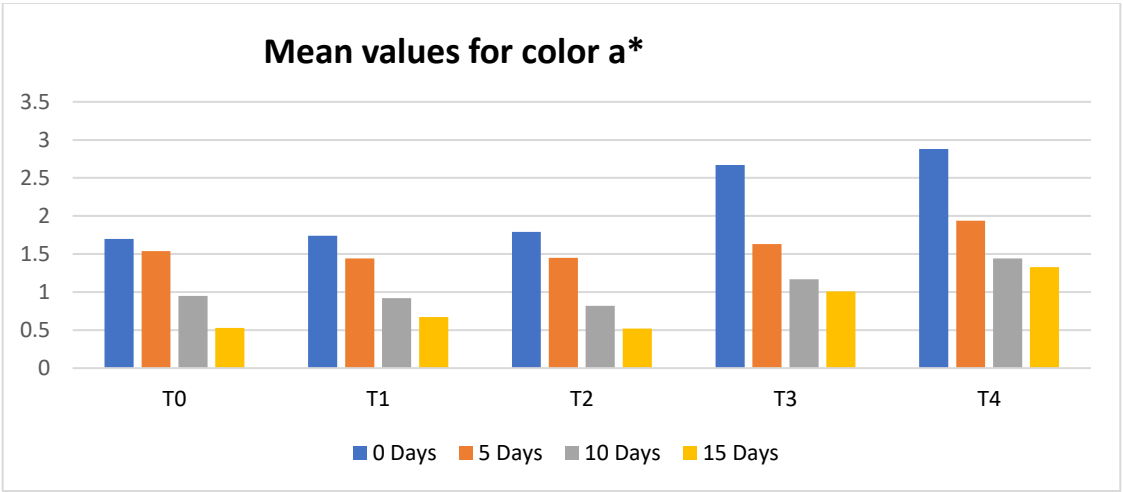
Mean values for Color (L*)



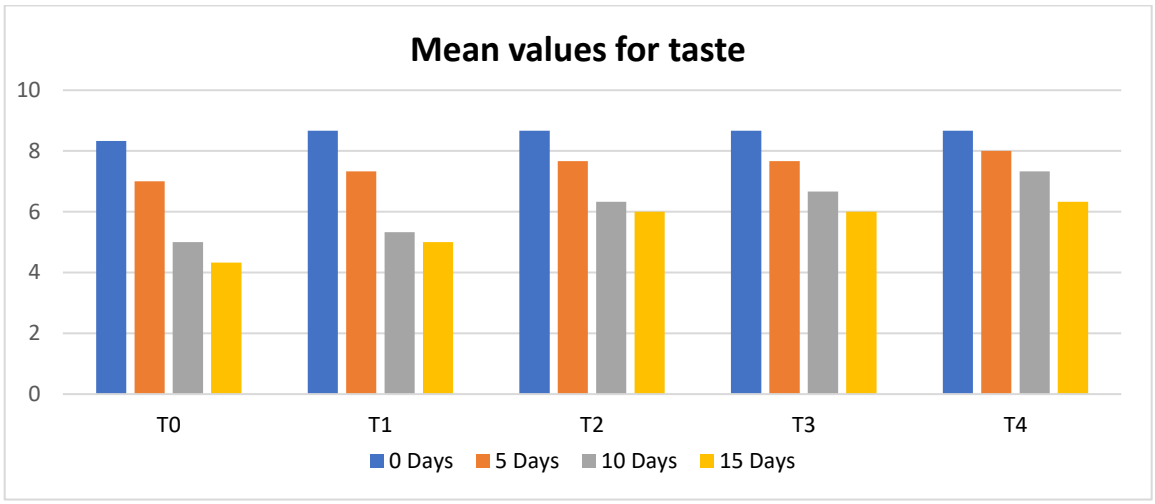
Mean values for Color (b*)



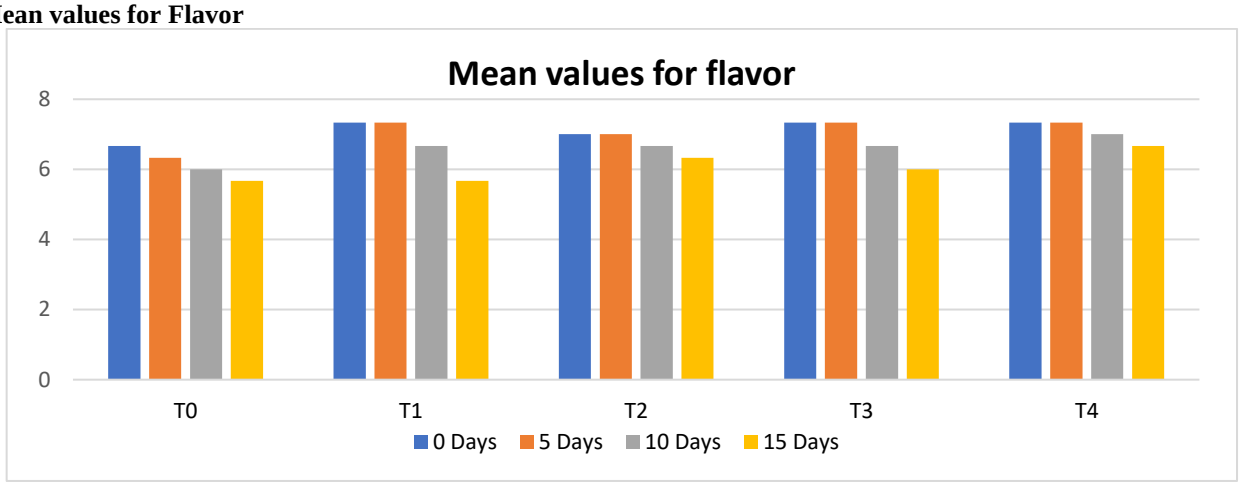
Mean values for Color a*



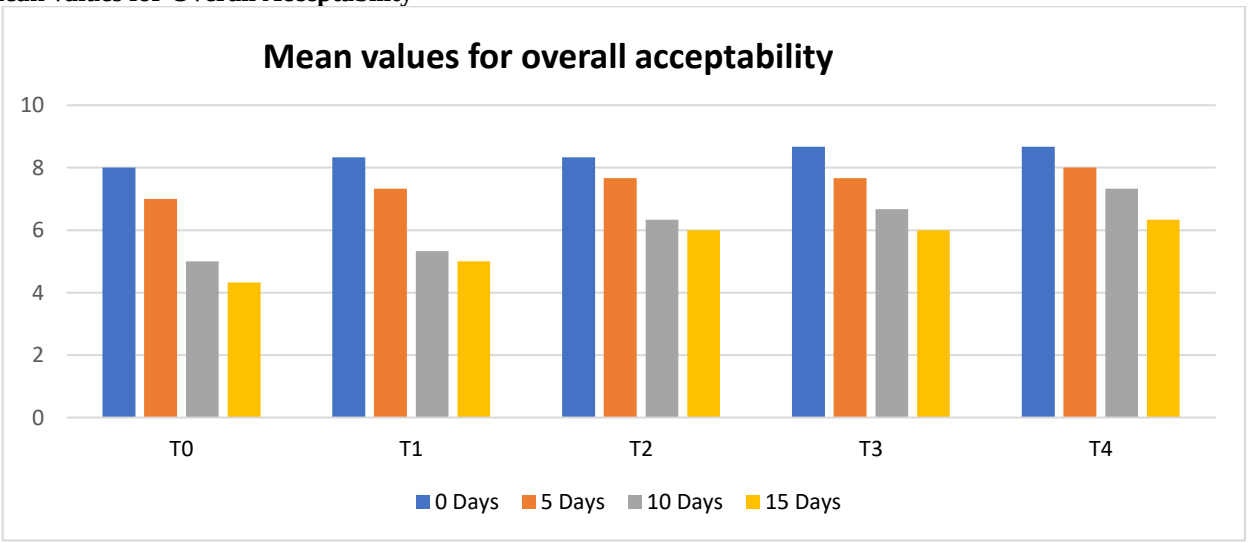
Mean values for Taste



Mean values for Flavor



Mean values for Overall Acceptability



Discussion

The average readings for the various treatments revealed that T_2 (0.43±0.06) had the highest level of titratable acidity, followed by T_4 (0.41±0.09), T_1 (0.38±0.06), T_3 (0.37±0.04) and the control sample (0.34±0.04). While the highest and lowest values recorded during the investigations were for T_2 and T_0 respectively. The average number of days for the titratable acidity of apple juice across the storage intervals shows that the minimum titratable acidity of 0.34 was found at 0 day and significantly increases over time, with the maximum titratable acidity being found at day 15 was (0.51). The pH of the juice wasn't significantly altered by nisin assisted thermosonication. The table contains the information obtained from apple juice research in which fresh apple juice was treated with nisin assisted thermosonication with the frequency

of 24kHz at temperature of 30 to 60°C for 50 min and addition of nisin at the concentration of 75 to 100 mg/L. The average readings for the various treatments revealed that T_0 (3.91±0.07) had the highest pH, followed by T_1 (3.88±0.09), T_3 (3.87±0.03), T_4 (3.83±0.04) and the T_2 (3.84±0.04). At 0 day, T_0 (3.91) and T_4 (3.83) were the highest and lowest levels recorded during the experiments. This pH level's lowest reading was recorded for T_4 after 15 days of storage. The average number of days for apple juice's pH throughout the course of storage intervals shows that the highest pH, 3.91, was recorded on day zero, and that pH significantly declines over time, with the lowest pH being recorded on day 15 at 3.66. The average number of days for the amount of total soluble solid in apple juice across the storage periods showed that the lowest amount of total soluble solid, 13.03, was found at day 0 and that the amount of total soluble solid that was found at day 15 was the highest 13.52. Costa *et al*. (2013) stated this outcome was consistent with which discovered an increase in TSS in apple juice. The overall interaction between treatments and the storage was also non-significant. The TSS value of sugarcane juice was also reported to rise over time by Ozyurt (2019).

The average readings for the various treatments revealed that T_0 (4.09±0.05) had the highest levels of ascorbic acid, followed by T_3 (3.90±0.07), T_1 (3.89±0.07), T_4 (3.68±0.04) and T_2 (3.67±0.08) at 0 day. The highest and lowest values recorded during the experiments were at T_0 (4.09±0.05) zero day and T_3 (3.90±0.07) respectively. The average number of days for ascorbic acid in apple juice over the course of storage intervals shows that the highest amount, (4.09) was found at day 0 and that ascorbic acid dramatically drops over time, peaking at day 15 with a value of 3.47. This decreasing trend with temperature and storage were comparable to those of Abid *et al*. (2014) in orange juice and Alves *et al*. (2015) in tangerine juice. They also discovered that the juice's ascorbic acid content reduced over the course of storage. Apple juice exhibits a comparable increase in phenolic concentration. Results on the impact of thermosonication on the total phenolic components of apple juice are shown in Table (4.5b). When compared to the controlled sample, ultrasound had the ability to raise the total phenolic contents but thermal treatment caused the phenolic contents to decrease as stated by Santhirasegaram *et al*. (2015b). The total phenolic contents of the sonicated samples were higher than those of the control sample. Mean results for several treatments at 0 day revealed that T_4 (291.6±0.46) had the highest total phenolic content, followed by T_2 (291.23±0.40), T_3 (285±0.46), T_1 (284.86±0.47) and the control sample (274.53±1.56). While the lowest and highest values recorded during the experiments were in T_0 at 15th day (253±1.56) and in T_4 maximum (291.6±0.46) at 0 day, respectively.

The treatment like TS significantly increases the cloud value of apple juice. The cloud value for apple juice in the control sample was 0.09. The results were 0.85 and 0.84 in T_1 and T_3 when the TS at 24 kHz treatment was applied for 50 minutes at 30°C. The cloud values were 1.001 and 1.13, respectively, even in the TS treatments (T_3 and T_4). With regard to apple juice, it can be inferred from the results that non-thermal technologies, such as TS and TS+NS, can raise the cloud value of the juice. Both of these treatments had a substantial ($p < 0.05$) impact on the difference between the maximum and minimum values T_4 (1.13 ± 0.05), and T_0 (0.09 ± 0.03) indicating that the cloud value of juices is influenced by the treatment's conditions, including processing time and temperature. The study's final results showed that, compared to the subsequent storage time of 15 days, there was minimal difference in the growth of total plate count before initial 5 days after the initial storage interval of 5 days the significant increase in the total plate count can be seen. The highly significant increase in the total plate count was found in control sample after the storage of 15 days. This increase is due to time storage time increased. After the treatment by thermosonicatin and TS+NS significant changes were found in T_4 and T_3, this is due to the addition of nisin in the thermosonically processed juice. T_4 and T_3 retained the and controlled the microbial count the most. Thus, the given study proved that nisin can increase the decline effects of thermosonication on the microbial count significantly and can retained these values during storage.

Color L* was improved after thermosonication treatment, which reversed the effects of thermal treatment, however it degraded over time while being stored. Addition of nisin to the sample showed non-significant effects. Alternatively, means are shown and data L* values range from the average readings for the various treatments revealed that T_4 (50.32±0.18) had the highest level of color L*, followed by T_2 (49.63±0.20), T_3 (49.42±0.19), T_1 (48.28±0.30) and T_0 (47.43±0.19) at zero day. The lowest and highest values recorded during the investigations were in T_0 at 15$_{th}$ day (42.43±0.12) and in T_4 maximum day 15 (50.32±0.18), respectively. The color was reduced by thermal treatment, improved by sonication treatment, but degraded over time while being stored. The average values for the various treatments revealed that T_4 (16.56±0.18) had the highest color b*, followed by T_3 (15.56±0.16), T_2 (13.85±0.19), T_1 (13.54±0.18) and the control sample (12.75±0.2). While the highest and lowest values recorded during the experiments were, respectively, during zero days of T_4 (16.56) and T_0 (10.12±0.12) at 15th day. The average number of days for the color b* of apple juice across the storage intervals shows that the highest value was found at day 0 and dramatically drops over time, with the lowest value, 10.12, being found at day 15. The greatest color a* was seen in T_4 (2.88±0.18), followed by T_3 (2.67±0.16). According to mean values for different treatments (Table 4.11b) the maximum and minimum value are of T_4 (2.88±0.18) and T_0 (1.70±0.12) respectively at the zero day.

The average readings for the various treatments revealed that T4 (6.83) had the highest level of flavor, followed by T_3, T_2, T_1 and T_0 (6.00). While the lowest and highest values were recorded at zero days for T_0 (6.67±0.58) and T_4 (7.33±0.58), respectively. The controlled sample T_0 (5.67±1.00) had the lowest flavor value after 15 days of storage. The average number of days for the flavor of apple juice across the storage periods shows that the highest flavor 7.33, was noticed at zero day (4.9b), and that flavor partially dropped over time, with the lowest flavor being noticed at 15 day (5.67), in particular. Mean values for various treatments showed that highly taste was observed in T_4 (7.58) and followed by T_3 (7.25), T_2 (7.17), T_1 (6.67) and T_0 (6.16). While the minimum and maximum values observed during the studies were at zero days of T_0 (8.33±0.58) and T_4 respectively. This lowest value of taste was recorded on the 15th day of storage in

controlled sample T_0 (4.33±0.12). Mean of days for the taste of pineapple juice throughout the storage intervals reveals that the maximum taste 8.69 was observed at zero day and with the passage of time decreases significantly and lowest taste was observed at 15th day 5.53 specifically. The concluded results of this study indicated that there is little change observed towards decrease in taste after first storage interval of 5 days as compare to next storage interval of 15 days. The average results for the various treatments revealed that T_4 (7.89) had the highest overall acceptance, followed by T_3 (7.0), T_1 (6.83), T_2 (6.74) and T_0 (6.42). At zero days of T_0 (8.00±0.58) and T_4 (8.67±0.33), respectively, were the least and greatest values recorded during the experiments. On the 15th day of storage, controlled sample T_0 (4.67±0.33) recorded the lowest result of overall acceptability. The average number of days for the apple juice's overall acceptability over the course of storage intervals shows that the highest color, 8.33, was found at day 0 and that it dramatically falls over time, with day 15 showing the lowest overall acceptability, at 5.20.

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