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Kinetics and effects of aeration and thermosonication on physiochemical, microbial and sensory properties of grape juice during storage periods

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Abstract
The present work is aimed to study the kinetics and effects of combination of both aeration and thermosonication on physiochemical, microbial and sensory properties of grape juice during storage periods of 90 days. The main objective of the present work is to increase the shelf life of the grape juice but without adding any chemical preservative. The grape juice was aerated for 30 minutes and thermosonicated at 60^0C at various time intervals of 5,10,15,20 and 30 minutes to determine the pH, TSS, Titratable acidity, Ascorbic acid, Reducing sugar, Microbial load, and Sensory Properties during storage periods. It was observed that the optimum condition for aerated grape juice was 20 minutes thermosonicated sample and it had pH of 3.9, TSS of 17.5 ºBrix, Titratable acidity of 0.84 (g/100ml), Reducing sugar of 10.26 (µg/ml), Ascorbic acid of 2.65 (mg/100g) Microbial load of 3 (CFU/ml), and Sensory score of 9. The shelf life of grape juice was enhanced up to 45 days by combination of both aeration and thermosonication techniques.

Keywords: grape juice, shelf life, titrtable acidity, reducing sugar

Introduction

Food products are perishable by nature and require protection from spoilage during their Preparation, storage and distribution to give them desired shelf life. Spoilage or other chemical changes lead to loss of shelf life, which may occur in any stages of raw materials or during consumption of the finished products. The principal reactions that leads to the spoilage are include physical, chemical, microbial and enzymatic factors. Microorganisms are the main agents which are responsible for food spoilage and food poisoning and therefore food preservation techniques are targeted towards them. Food preservation methods are currently used by the industry on the inhibition of microbial growth or on microbial inactivation. Methods which prevent or slow down microbial growth cannot completely declare food safety (Manas and Pagan, 2005) [5]. The trend of production of prolonged shelf life and convenient foods which are fresh like is increased nowadays by consumers. For prolonged shelf life, the main aim is efficient inactivation of microorganisms and enzymes for effective preservation.

It is well established that traditional thermal food pasteurization and sterilization techniques can extend the shelf life of food products and ensure their safety, but they cause loss in valuable nutritional and physicochemical parameters. In conventional heating methods, food products are heated externally. The time required to increase the temperature at cold point may lead to over processing of the product. And this over processing results in destruction of nutrients and flavors of the product and it has low energy efficiency. To overcome these disadvantages innovative and novel technologies are researched for adaption in practical use. In order to overcome the problems, there is a need to introduce novel technologies that can successfully be used to extend shelf life, ensure safety, improve quality and consumer acceptance without any adverse effect and damage to the nutrients.

Non-thermal methods such as addition of natural antimicrobial agents in food, high pressure processing, ultrasonication, ozone, pulsed electric field, and ultraviolet is increasingly gaining attention for food processing and preservation.

Ultrasonication is such an innovative and non-thermal technique which prevents losses from the product but also improves the ultimate nutritional quality of product and reduces the microbial load in foods. Ultrasound techniques have application in both the analysis and modification of foods. It is considered to be more advantageous due to its reduced processing time, with low energy consumption, enhanced quality, reduced chemical and physical hazards, improve the shelf life and being environmental friendly (Abid *et al*., 2013) [1].

Materials and Methods

Sample preparation

The ripened grapes were purchased from the market. The grapes were washed in clean water to remove the adhered debris present on the skins of the grapes. The washed grapes are blanched at 90 ^{0}C for 2 min to deactivate the enzyme. After blanching, the grapes was crushed in the mixer grinder and the grape juice was extracted and filtered in the filter cloth to remove suspended solid particles such as skins and seeds etc. The grape juice was filtered for three times to ensure that the juice is free from suspended solid particles. The clear clarified grape juice of 100 ml is taken for the experimental study. Each experiment was conducted in triplicate and the average values were taken.

Experimental Design

- Independent variable: Temperature, Aeration and time combination.
- Dependent variable: pH, TSS, titratable acidity, microbial load, ascorbic acid, reducing sugar, and sensory analysis.
- The frequency is constant (50 Hz) and based on various

trials the treatment time was fixed as 5, 10, 15, 20, and 30.
- The temperature chosen for thermosonication treatment was 60 ºC.
- The aeration time was fixed as 30 min.

Ultrasonic Bath

Ultrasonic cleaning system consists of stainless steel jacketed vessel, sonication bath, frequency, time, and time control system, and cooling system shown in the figure 3.1. An ultrasound generating transducer built inside the chamber, or lowered into the fluid, produces ultrasonic waves in the fluid by changing size in concert with an electrical signal oscillating at ultrasonic frequency. An aqueous (water) or organic solvent, depending on the application was used as an ultrasonic waves transfer medium.

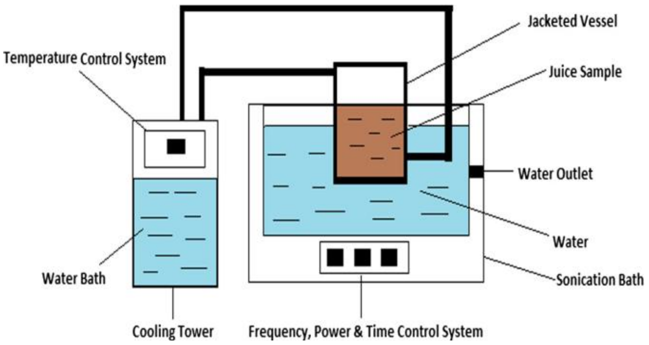

Fig 1: Schematic diagram of ultrasonic cleaning (Bath) system

Methods

The clear clarified grape juice was kept in 4-5 ^{0}C in the refrigerator for 5 hours. The grape juice was aerated with air through air pump for 30 min. The aerated grape juice was thermosonicated at 50 HZ frequency and temperature was maintained at 60 ^{0}C for various time intervals of 5,10,15,20 and 30 min. The treated and untreated grape juice was tightly packed and kept at 4-5 ^{0}C in the refrigerator. The sample was tested for physiochemical, microbiological and organoleptic properties on storage periods of 15, 30, 45, 60, 75 and 90 days.

Treatment Process

All lots of samples contain 100 ml were transferred to a glass beaker. The juice samples were sonicated in ultrasonic cleaning bath. Grape juice was aerated for 30 min and then followed by thermosonication at 60 ºC. The equipment has frequency about 50 Hz. The variable parameters are temperature, aeration and treatment time. The sample containing beaker was placed in the inner tank of ultrasonic cleaning bath. After the initial trials the treatment time combinations were fixed. The samples were processed at constant frequency of 50 Hz and the power range of 1500 W was used. The treated and untreated grape juice was then filled in pet bottles. The bottles were stored at refrigerated condition (4 to 5 ºC). They were analyzed physiochemically (pH, total soluble solids, acidity, ascorbic acid, and reducing sugars), microbiologically and organoleptically (color, flavor, taste and overall acceptability) for total period of 90 days at 15 days interval.

Analysis of Fresh and Ultrasonicated Samples PH

PH is a measure of the acidity of an aqueous solution. PH was determined using pH meter. It is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution. The pH meter was standardized with buffer solution. Then the probe was immersed into the sample and readings were displayed digitally (Tarazona, 2013) [10].

Total soluble solid

TSS is the sum of the solids present in a solution. As it increases, water activity is reduced and survival of microorganisms becomes less likely (Tarazona, 2013) [10]. TSS was determined using optical refractrometer. A drop of the sample was placed on the prism and readings were taken.

Titratable acidity

Acidity is the measure of level of acid present in a solution. Acidity depends upon on microbial load. The end point was attained when the sodium hydroxide neutralizes the weak acid present in the juice. It was determined by titrating the sample against sodium hydroxide and using phenolphthalein as an indicator (Tarazona, 2013) [10]. Pasteurized samples are titrated against sodium hydroxide and the acidity is expressed as tartaric acid. The formula for determining the Titratable acidity

$$\text{Titratable acidity (g/100ml)} = \frac{\text{Titre value} \times \text{normality of NaOH} \times \text{Acid factor}}{10 \text{ (ml of sample)}}$$

Where, acid factor is based on type of juice. The acid factor of grape juice is 0.075.

Reducing sugar

Reducing sugars like glucose, lactose, galactose, treated with dinitrosalicylic acid (DNS) reagent then it is reacted with Rochelle salt (potassium sodium tartarate salicylate), the concentration is measured in calorimeter at 510nm. The absorbance was noted at 510nm (Miller, 1959) [6].

Ascorbic acid

Ascorbic acid is a naturally occurring compound with antioxidant properties. It is determined using Indophenol method. The dye is first standardized with ascorbic acid. After standardization the 5ml of sample is titrated against the dye with the dilution of 5ml of water and 5ml of (Tee *et al*., 1988) [11].

Ascorbic acid (mg per 100g) =

$$\frac{Normality\ of\ ascorbic\ acid \times titre\ value \times 100}{Volume\ of\ the\ sample}$$

Microbial Load

Microbial count was found using plate count method. Pasteurized samples at various temperatures are taken and count of the microbial load is taken. Plate count agar was used as a medium for microbial analysis at 10^{-5} dilution. Serial dilution was done microbial load had been analyzed for determining bacteria, yeast, fungal growth. (Tarazona, 2013) [10].

Sensory analysis

Sensory evaluation is performed by Affective or Hedonic Test. This method is useful for measuring food acceptability. It uses a 9 point Hedonic scale ranging from 'extremely dislike' to 'extremely like' (Van Aardt *et al.*, 2001) [12]. Initially and periodically, sensory characteristics of all samples were evaluated for different sensory attributes by a panel of 5 panelists. All the panelists were briefed before evaluation. Sensory attributes like appearance and color, aroma, taste and overall acceptability for all samples were assessed using nine point hedonic scales.

Kinetics on degradation of ascorbic acid

The degradation of ascorbic acid in grape juice upon storage period was evaluated using first-order kinetic model. The first order reaction is

$\ln [A] = \ln [A]_0 - kt$

The equation has the form of the algebraic equation for a straight line, $y = mx + b$, where $y = \ln[A]$ and $b = \ln[A]_0$, the graph was plot of ln[A] versus t for a first-order reaction should give a straight line with a slope of $-k$ and an intercept of $\ln[A]_0$ (Sapei and Hwa, 2014) [9].

Result and Discussion

Effect of Storage on Ph

There was a gradual decrease in pH content of aerated and thermosonically treated samples was shown in the figure 2. The pH value decreases for all samples. Maximum decrease was observed for the untreated sample from 3.9 to 2.9. Minimum decrease of pH content was observed for 20 min aerated and thermosonicated treated samples from 3.9 to 3.7. The pH content of 20 min treated sample significantly decreased after 45 days of storage. The satisfactory result was obtained for 20 min aerated and thermosonicated sample.

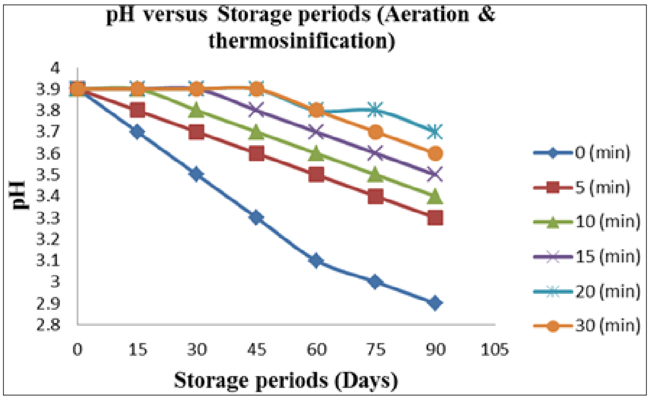

Fig 2: Effect of aeration and thermosonication on pH during storage

Effect of Storage on Total Soluble Solids

There was a slight increase in TSS of the samples was shown in the figure 3. Highest increase was observed for the untreated sample from 17.5 to 19.2 ^{0}Brix. Minimum increase of TSS content was observed for 20 min aerated and thermo sonically treated samples from 17.5 to 17.8 ^{0}Brix. The TSS content of 20 min treated sample was significantly increased after 45 days of shelf life. Increase in TSS during storage was also reported by (Rani *et al.*, 2012) [8] for grape juice preserved by chemical preservatives.

Minimum increase of TSS was found in aeration and thermosonification of grape juice. Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj and Pandey 2011) [3].

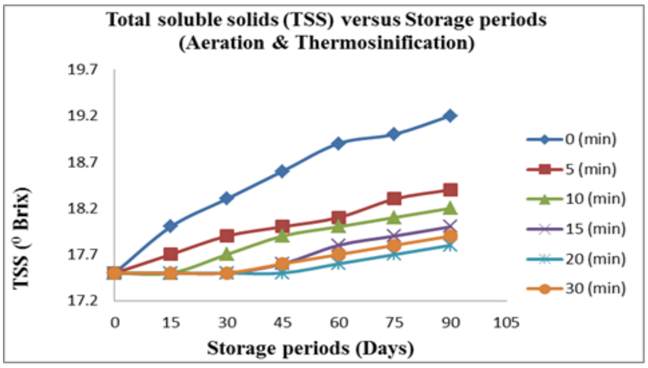

Fig 3: Effect of aeration and thermosonication on TSS during storage

Effect of Storage on Titratable Acidity

The acidity shows increasing trend during storage and it was shown in the figure 4. Highest increase in acidity content was observed in untreated sample upto 1.02 g/100ml. The minimum increase of acidity was found in 20 min aerated and thermosonicated sample upto 0.86 g/100ml. The changes were observed after 45 days. Treated samples had lower acidity which indicated lower microbial growth compared with fresh juice. The same increasing trend in acidity was also reported by (Ayub and Bilal, 2001) [2] in pomegranate syrup sample.

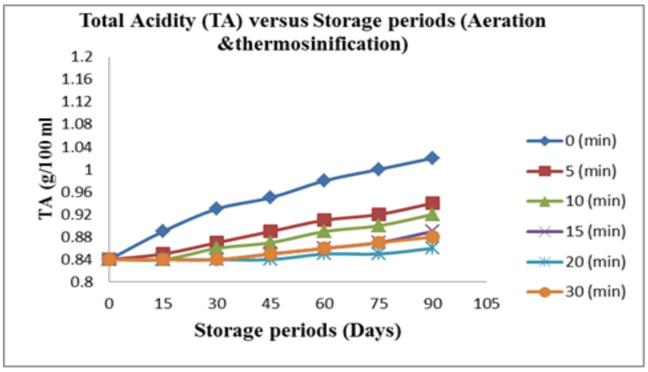

Fig 4: Effect of aeration and thermosonication on TA during storage

Effect of Storage on Reducing Sugar

There was a gradual increase in RS content of samples was shown in the figure 5. The highest increase was observed for untreated samples from 10.2 to 13 µg/ml. And lowest increase was observed for 20 min aerated and thermosonicated samples from 10.2 to 10.5 µg/ml. The change in RS was observed for 20 min samples after 45 days. The same increase in reducing sugars content was reported by (Wisal *et al.*, 2013) [13] in strawberry juice samples.

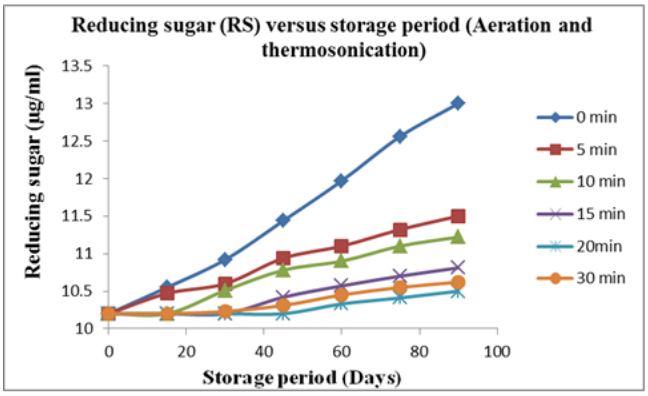

Fig 5: Effect of aeration and thermosonication on RS during storage

Effect of Storage on Ascorbic Acid

The effects of aeration and thermosonication on the ascorbic acid of grape juice during storage were depicted in Figure 6. During storage ascorbic acid content was decreased gradually. The maximum decrease was observed in untreated sample from 2.71 to 1.12 mg/100 g. Minimum decrease was observed in 20 min aerated and thermosonically treated sample from 2.71 to 2.49 mg/100g. Aeration and thermosonication treatment shows no such significant changes upto 45 days for 20 min treated samples. Decrease in ascorbic acid content was also reported by (Kinh *et al*., 2001) [4] in grape juice samples. High ascorbic acid retention was attained in aeration and thermosonicated sample at 20 min.

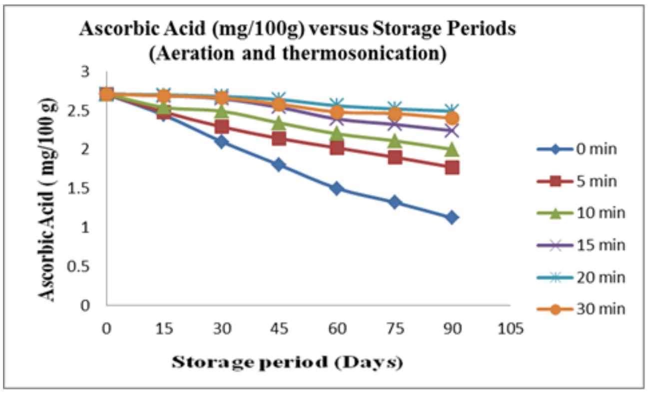

Fig 6: Effect of aeration and thermosonication on ascorbic acid during storage

Effect of Storage on Microbial Load

After aeration and thermosonication treatment there was decrease in microbial count was observed in all samples was shown in the figure 7. The highest decrease was observed in 20 and 30 min. lowest decrease was observed in 5 and 10 min treated samples. During storage increasing pattern of microbial load was observed. The highest increase was observed in untreated sample upto 453 CFU/ml. Minimum increase of microbial population was observed in 20 min aerated and thermosonically treated samples from 3 to 13 CFU/ml. The increasing trend was observed after 45 days of storage in 20 min treated sample.

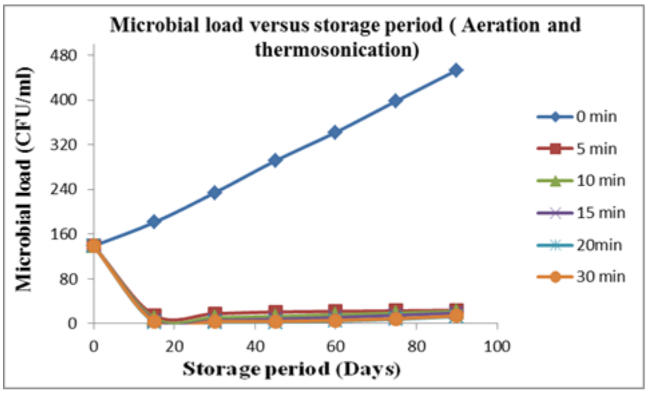

Fig 7: Effect of aeration and thermosonication on microbial load during storage

Effect of Storage on Overall Acceptability of Processed Juice

There was a gradual decrease in overall acceptability score of all samples was shown in the figure 8. Highest decrease in overall acceptability score was observed in untreated sample from 9 to 1 and minimum in 20 min from 9 to 8.6. The change in overall acceptability was observed after 45 days in aeration and thermosonically treated product. The high retention in sensory properties was achieved in aeration and thermosonication treated grape juice. Prasad and Mali, (2000) reported that the colour, flavour and taste of the pomegranate squash remained better at low temperature than at room temperature.

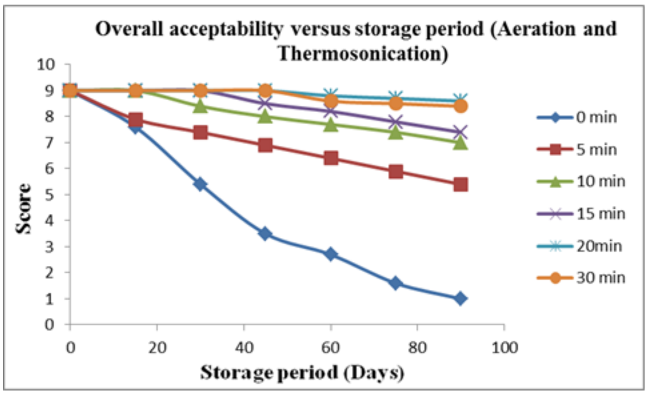

Fig 8: Effect of aeration and thermosonication on overall acceptability during storage

Kinetic Studies on Ascorbic Acid Degradation during Storage Periods

The first order kinetics was fit to the ascorbic acid degradation during storage periods of 90 days and corresponding with consideration of R^2 value and K were shown in the table 1. The ln (A) values for untreated sample and combination of both aeration and thermosonication of grape juice sample of various treatment was shown in table 2. The ln (A) versus storage period days plot was shown in fig (9-14).

Table 1: Experimental values of Ascorbic acid content (mg/100 g)

Time (Min)	Storage Period (Days) Ascorbic acid content (mg/100 g)							K values	R^2 values
	Fresh	15	30	45	60	75	90		
0	2.71	2.44	2.1	1.8	1.5	1.32	1.12	0.0096	0.9947
5	2.71	2.48	2.29	2.14	2.02	1.9	1.77	0.0049	0.9887
10	2.71	2.54	2.49	2.34	2.2	2.11	2	0.0034	0.9920
15	2.71	2.69	2.65	2.54	2.39	2.32	2.24	0.002	0.9316
20	2.71	2.7	2.68	2.64	2.56	2.52	2.49	0.0009	0.9336
30	2.71	2.69	2.66	2.58	2.48	2.46	2.4	0.0013	0.9530

Table 2: Shows ln (A) values for various storage periods

Storage Period (Days)	ln(A) 0 min	ln(A)5 min	ln(A)10 min	ln(A)15 min	ln(A)20 min	ln(A)30 min
0	1.00	1.00	1.00	1.00	1.00	1.00
15	0.85	0.92	0.95	0.97	0.98	0.98
30	0.71	0.85	0.89	0.94	0.97	0.96
45	0.56	0.78	0.84	0.91	0.96	0.94
60	0.42	0.70	0.79	0.88	0.94	0.92
75	0.28	0.63	0.74	0.85	0.93	0.90
90	0.13	0.56	0.69	0.82	0.92	0.88

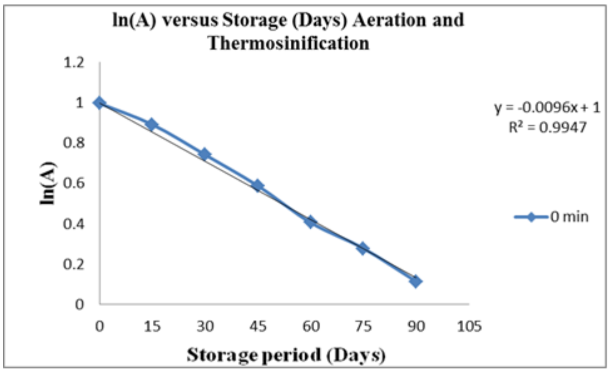

Fig 9: Shows plot of ln (A) versus Storage Periods at 0 min

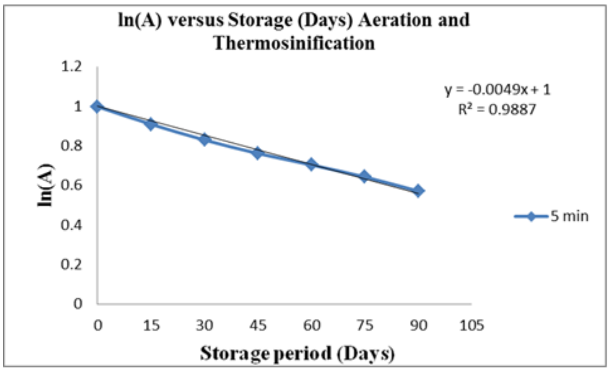

Fig 10: Shows plot of ln (A) versus Storage Periods at 5 min

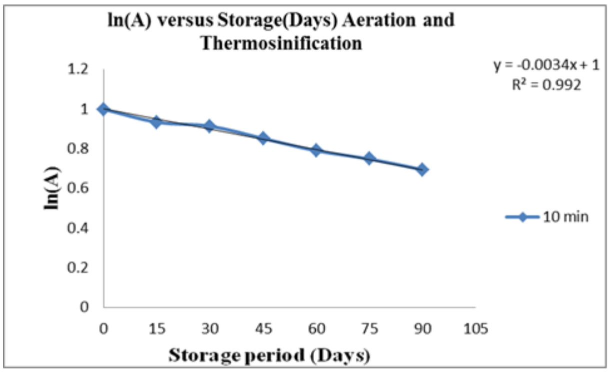

Fig 11: Shows plot of ln (A) versus Storage Periods at 10 min

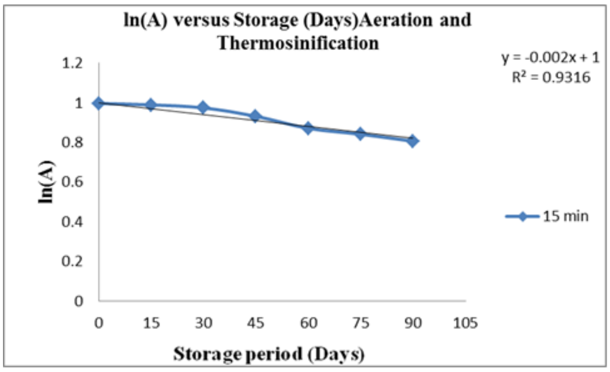

Fig 12: shows plot of ln (A) versus Storage Periods at 15 min

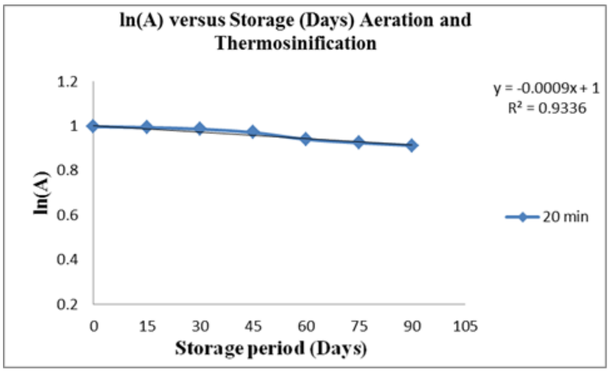

Fig 13: Shows plot of ln (A) versus Storage Periods at 20 min

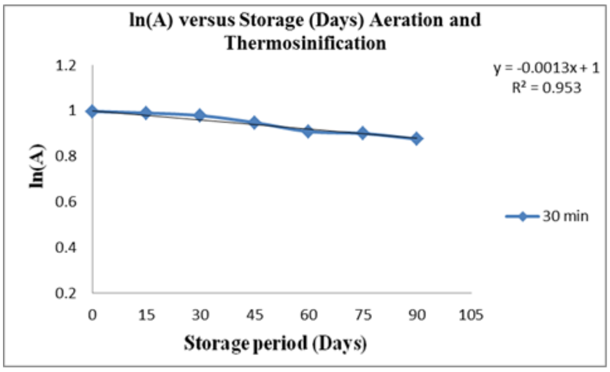

Fig 14: shows plot of ln (A) versus Storage Periods at 30 min

Table 3: Shows the Predicted values of Ascorbic acid content (mg/100 g) for various storage periods

Storage Period (Days)	A(0 min)	A 5 min	A 10 min	A (15min)	A(20 min)	A(30 min)
0	2.71	2.71	2.71	2.71	2.71	2.71
15	2.35	2.52	2.58	2.63	2.67	2.66
30	2.03	2.34	2.45	2.55	2.64	2.61
45	1.76	2.17	2.33	2.48	2.60	2.56
60	1.52	2.02	2.21	2.40	2.57	2.51
75	1.32	1.88	2.10	2.33	2.53	2.46
90	1.14	1.74	2.00	2.26	2.50	2.41

Table 4: Shows the comparison of Experimental and Predicted values of Ascorbic acid content (mg/100 g) for various storage periods

Time (Min)	Experimental values Storage Period (Days) Ascorbic acid content (mg/100 g)							Predicted values Storage Period (Days) Ascorbic acid content (mg/100 g)					
	Fresh	15	30	45	60	75	90	15	30	45	60	75	90
0	2.71	2.44	2.1	1.8	1.5	1.32	1.12	2.35	2.03	1.76	1.52	1.32	1.14
5	2.71	2.48	2.29	2.14	2.02	1.9	1.77	2.52	2.34	2.17	2.02	1.88	1.74

10	2.71	2.54	2.49	2.34	2.2	2.11	2	2.58	2.45	2.33	2.21	2.10	2.00
15	2.71	2.69	2.65	2.54	2.39	2.32	2.24	2.63	2.55	2.48	2.40	2.33	2.26
20	2.71	2.7	2.68	2.64	2.56	2.52	2.49	2.67	2.64	2.60	2.57	2.53	2.50
30	2.71	2.69	2.66	2.58	2.48	2.46	2.4	2.66	2.61	2.56	2.51	2.46	2.41

The experimental and predicted values are shown in the table 4. There was no significant difference was observed between experimental and predicted values. The experimental and predicted values of ascorbic acid degradation during various storage period was shown in figure 15 and 16. The optimized time for combination of aeration and theromosonication treatment of grape juice was observed at 20 min and its corresponding rate constant (K) and R^2 values were 0.0009 and 0.9336.

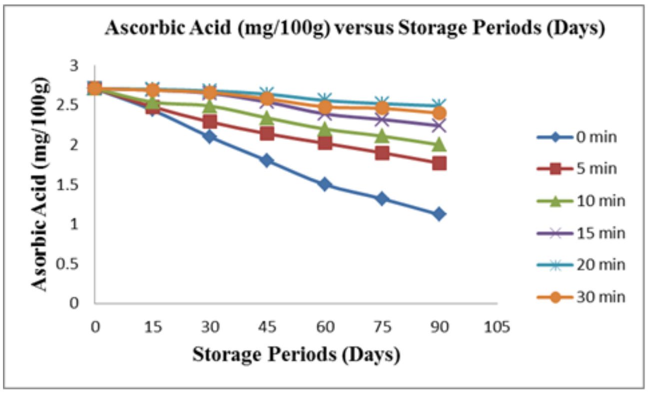

Fig 15: Experimental values of degradation of Ascorbic acid for various storage periods

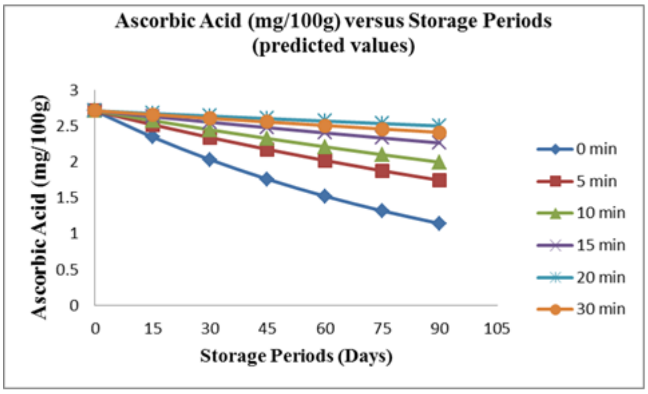

Fig 16: Predicted values of degradation of Ascorbic acid for various storage periods

Conclusion

From this study the effect of aeration and thermosonication treatment on quality of grape juice during storage period of 90 days was studied. It was concluded that aeration & thermosonication treatments on grape juice strongly affect the products shelf life and consumer acceptability. It was observed that the optimum treatment of combination of aeration and thermosonication for grape juice was 20 min for 45 days and it had pH of 3.9, TSS of 17.5, Titratable acidity of 0.84 (g/100ml), reducing sugar of 10.26 (µg/ml), Ascorbic acid of 2.65 (mg/100g), microbial load of 3 (CFU/ml)and sensory score of 9. The results suggest that the combination of aeration and thermosonication treatment on grape juice could improve its quality and shelf life of grape juice up to 45 days.

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