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Kinetics and Effects of Ultrasonication 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 ultrasonication 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 ultrasonicated at room temperature 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 treatment for grape juice was 20 min ultrasonicated sample for 15 days and it had pH of 3.9, TSS of 17.6°Brix, Titratable acidity of 0.84 (g/100ml), reducing sugar of 10.3 (µg/ml), ascorbic acid of 2.65 (mg/100g) microbial load of 10 (CFU/ml), and sensory score of 9. According to FDA, 5 log reductions in microbial load were attained 20 and 30 min ultrasonicated juice samples. The shelf life of grape juice was increased upto 15 days. The results suggest that ultrasound treatment technology could be potentially employed for the processing of grape juice and could improve its quality and safety.

Key words : Ultrasonication, Grape juice, pH, Ascorbic acid, 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). 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.

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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).

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 was blanched at 90 °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.

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 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.

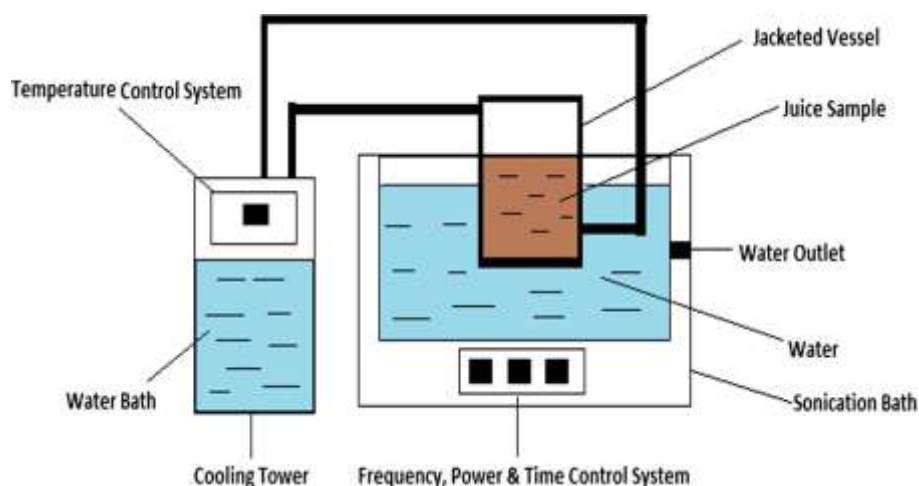


Fig.1 Schematic diagram of ultrasonic cleaning (Bath) system

Methods

Ultrasonication

The clear clarified grape juice was ultrasonicated at 50 Hz frequency and at various time intervals of 5,10,15,20 and 30 min. The ultrasonicated grape juice was tightly packed and kept at 4-5 °C in the refrigerator. The sample was tested for physiochemical, microbiological and sensory properties on storage periods of 15, 30, 45, 60, 75 and 90 days.

Experimental procedure

All lots of samples contain 100 ml of grape juice were transferred to a glass beaker. The juice samples were sonicated in ultrasonic cleaning bath. Grape juice was treated by ultrasonication at room temperature at various time intervals. The equipment has frequency about 50 Hz. 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 juice was then filled in pet bottles. The bottles were stored at refrigerated condition (4 to 5 °C). 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-Diaz, M. P. and Aguayo, E 2013).

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-Diaz, M. P. and Aguayo, E 2013). TSS was determined using optical refractometer. A drop of the sample was placed on the prism and readings were taken.

Titrateable 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-Diaz, M. P. and Aguayo, E 2013). Pasteurized samples are titrated against sodium hydroxide and the acidity is expressed as tartaric acid. The formula for determining the Titrateable acidity

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

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).

Ascorbic acid

Ascorbic acid is a naturally occurring compound with antioxidant properties. It is determined by 2, 6-dichlorophenol indophenol (DCPIP) titration method (Rao, B. and Deshpande, V., 2006). 5 mL of the ascorbic acid working standard (500µg/5 mL) and 10 mL of 4% oxalic acid were pipetted out into a 100 mL conical

flask. The contents in the flask were titrated against the dye solution (V1) until the appearance of a pale pink colour that persisted for a few min. 5 mL of the test sample was similarly titrated against the dye solution (V2). Ascorbic acid content present in the test samples were determined using the formula (Dinesh *et al.*, 2015):

$$\text{Ascorbic acid (mg per 100g)} = \frac{0.5\text{mg}}{V_1} \times \frac{V_2}{5\text{mL}} \times \frac{25\text{ mL}}{\text{Wt. of the sample}} \times 100$$

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-Diaz, M. P. and Aguayo, E, 2013).

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). 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).

Results and Discussion

Effect of Storage on pH

There was a gradual decrease in pH content of ultrasonically treated samples was shown in the (Figure 2). The pH value decreases for all samples. The Highest decrease was observed for the untreated sample from 3.9 to 2.9. The lowest decrease of pH value was observed for 20 min ultrasonically treated samples from 3 to 3.5. The pH content of 20 min treated sample significantly decreased after 15 days of shelf life. The pH change was may due to increase in the acidity of the juice. Decrease in pH during storage was also reported by (Imran *et al.*, 2000) in guava pulp samples.

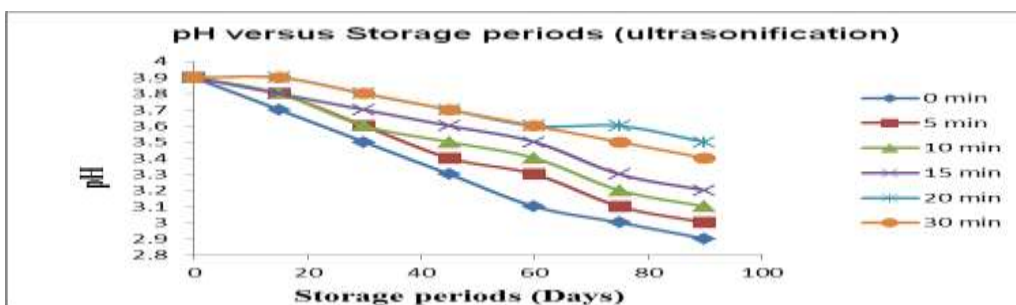


Fig.2 Effect of ultrasonication on pH during storage

Effect of Storage on Total Soluble Solids

The initial TSS of 17.5 was observed. There was a slight increase in TSS of the samples was shown in the (Figure 3). The TSS value increases for all samples. Highest increase was observed in the untreated sample from 17.5 to 19.2 and it may due to the conversion of non-reducing to reducing sugars. Minimum increase of TSS content was observed for 20 min ultrasonically treated samples from 17.5 to 18.4. The TSS content of 20 min treated sample significantly increased after 15 days of shelf life. The increase in TSS during storage for several varieties of citrus fruit was observed (Purvis, 1983).

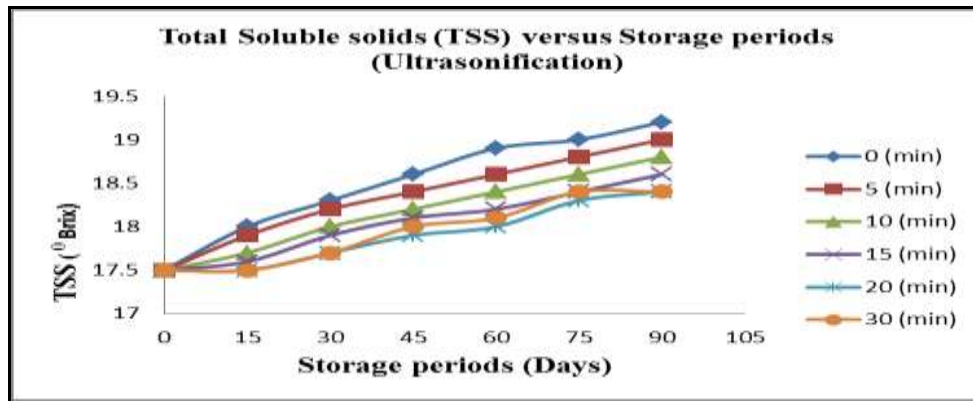


Fig. 3 Effect of ultrasonication on TSS during storage

Effect of Storage on Titratable Acidity

The titratable acidity increases during storage period was shown in the (Figure 4). The initial acidity of grape juice was 0.84. Highest increase in acidity content was observed in untreated sample from 0.84 to 1.04 and minimum increase was observed for 20 min ultrasound treated product upto 0.95. The changes occurred after 15 days in 20 min sample. The ultrasound treatment can delay the degradation of organic acids (Almenar et al. 2007) and thus can maintain the acidity and pH of fruits during storage periods. This trend of increase in titratable acidity is similar to the results of (Ziena, 2000).

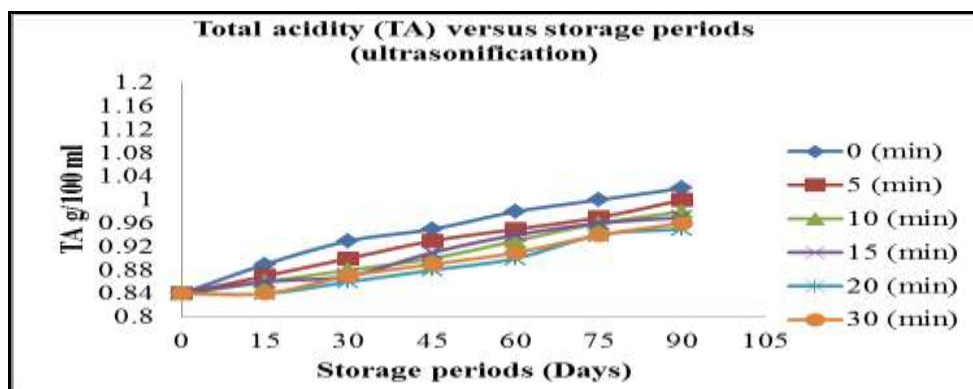


Fig. 4 Effect of ultrasonication on TA during storage

Effect of Storage on Reducing Sugar

Sugars are one of the most important constituents of fruit products, essential for and also act as a natural food preservative (Bhardwaj and Pandey, 2011). The results show a slight increase in RS with increasing storage period was shown in the (Figure 5). The sugar content of fruit juices usually increases with increased storage period. The highest increase was observed for untreated sample from 10.2 to 13. The minimum increase was observed at 20 min ultrasonically treated product 11.7. The increase in RS was observed after 15 days of storage in 20 min treated sample. The increase is probably due to the hydrolysis of polysaccharides like starch, cellulose, pectin, etc. and conversion into simple sugars (glucose, fructose). Kausar *et al.*,(2012) reported increased RS with increased storage time of a cucumber–melon functional drink.

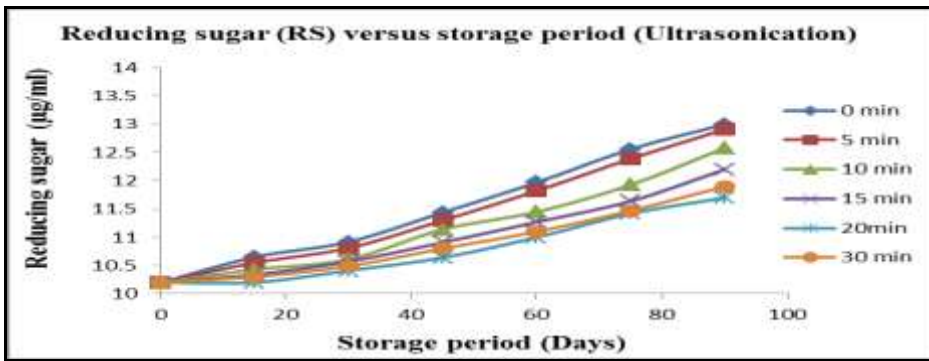


Fig. 5 Effect of ultrasonication on RS during storage

Effect of Storage on Ascorbic Acid

The effects of ultrasonication on the ascorbic acid of grape juice during storage were depicted in the (Figure 6). Ascorbic acid results have shown significant changes during storage period. During storage ascorbic acid content was decreased gradually. The maximum decrease was observed in untreated sample from 2.71 to 1.12. Minimum decrease was observed in 20 min ultrasonically treated product from 2.71 to 1.52. Decrease in ascorbic acid content was also reported by (Mehmood *et al.*, 2008) in his work on apple juice samples. Ultrasonic treatment shows no such significant changes upto 15 days for 20 min treated samples. Low temperature can slow down the rate of degradation of vitamin C generally. High losses seen in juice samples may be as result of oxidation reaction by residual oxygen, followed by decomposition which may have been accelerated due to storage temperature (Burdule *et al.*, 2006).

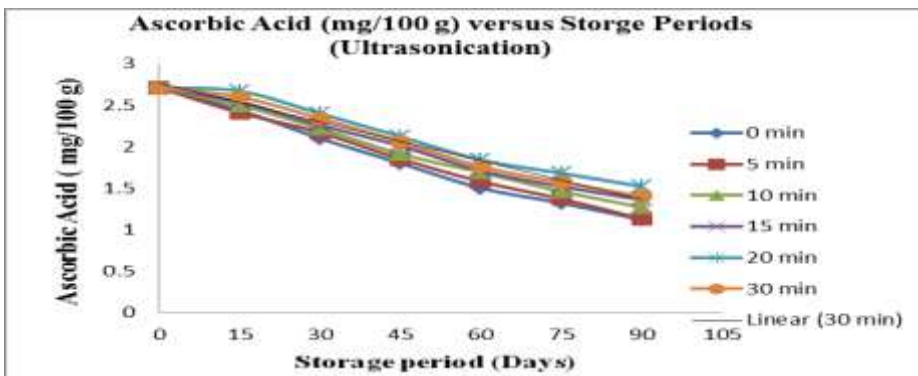


Fig. 6 Effect of ultrasonication on ascorbic acid during storage

Effect of Storage on Microbial Load

It was observed that there was a significant reduction in total plate count of all the samples sonicated for 5, 10, 15, 20 and 30 min as compared to non sonicated sample shown in the (Figure 7). A similar result was obtained by Abid *et al.* (2013) for ultrasound treated apple juice. The longer the ultrasound treatment time was the more microbes inactivated, which suggested that extending ultrasound treatment time could decrease the microbial load to a greater extent. During storage microbial population was increased. The highest increase was observed in untreated sample from 139 to 453 CFU/ml. In ultrasonically treated grape juice samples the increase of microbial population was observed minimum. The lowest increase was observed in 20 min ultrasonically treated product. Ultrasonic treatment reduces initial microbial to 8 CFU/ml in 20 min treated sample.

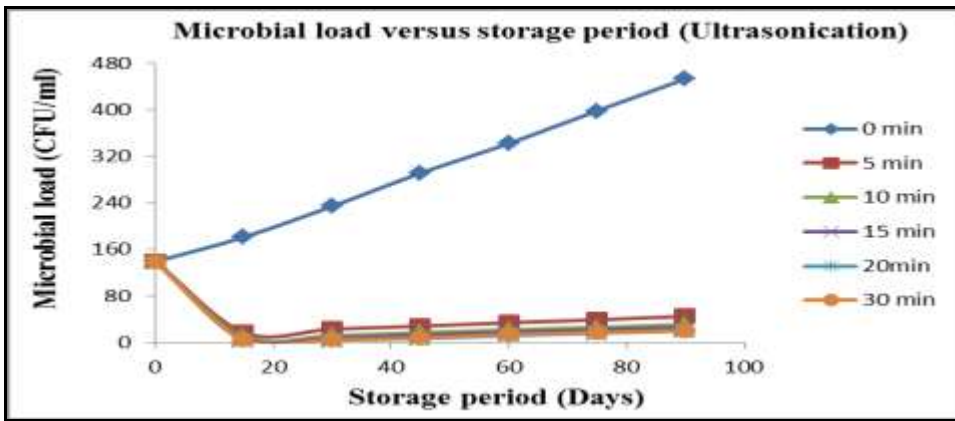


Fig. 7 Effect of ultrasonication on microbial load during storage.

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 6.1. The change in overall acceptability was observed after 15 days in ultrasonically treated product. The change in might be attributed to oxidation of phenolic compounds present, increase in acidity and some chemical reactions in juice. The same declining trend in overall acceptability score were shown by (Khan *et al.*, 2015) in orange juice stored in glass bottles.

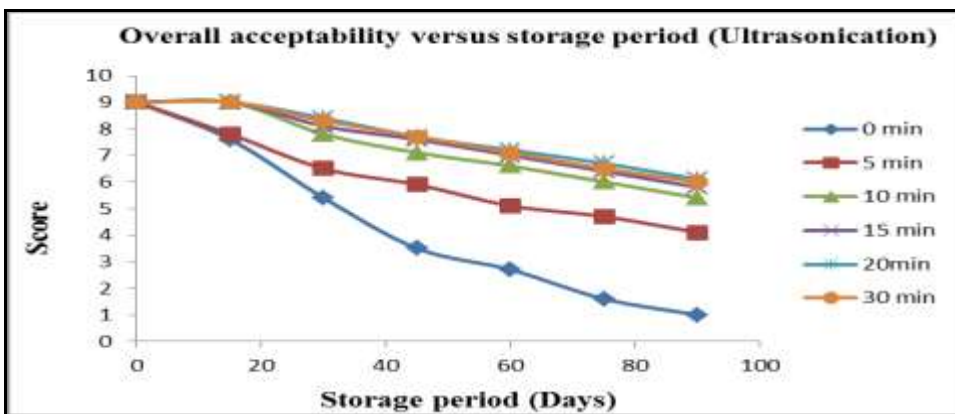


Fig. 8 Effect of ultrasonication on overall acceptability during storage

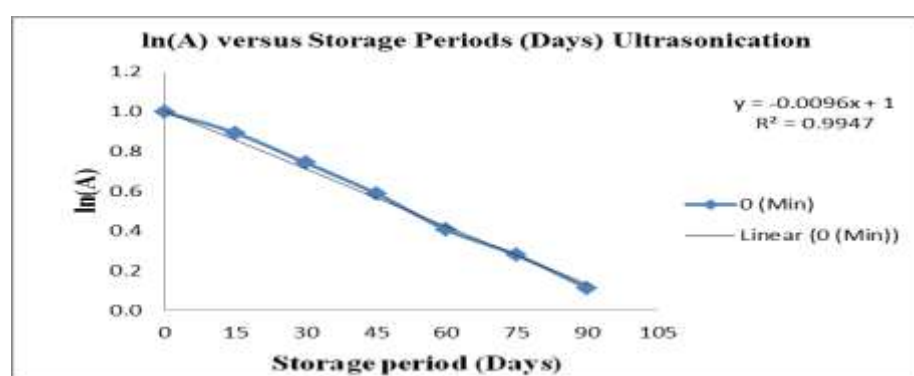
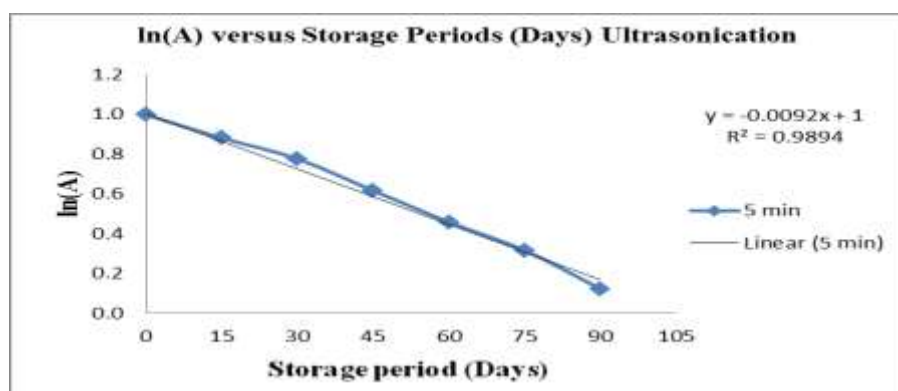
Kinetic Studies on Ascorbic Acid Degradation During Storage Periods

The first order kinetics was fit to the ascorbic acid degradation during storage periods of 90 days and corresponding R^2 and K values were shown in the table 1. The $\ln(A)$ values for untreated and ultrasonicated grape juice of various treatment time was shown in table 2. The $\ln(A)$ versus storage period days plot was shown in figure (9-14).

Time (Min)	Storage Period (Days)							K values	R^2 values
	Ascorbic acid content (mg/100 g)								
	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	2.41	2.17	1.85	1.58	1.37	1.13	0.0092	0.9894
10	2.71	2.5	2.22	1.91	1.69	1.46	1.27	0.0081	0.9904
15	2.71	2.54	2.26	2.01	1.71	1.52	1.36	0.0075	0.9860
20	2.71	2.67	2.4	2.12	1.84	1.68	1.52	0.0062	0.9632
30	2.71	2.6	2.34	2.06	1.76	1.57	1.4	0.0071	0.9729

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

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	0.86	0.88	0.88	0.90	0.89
30	0.71	0.72	0.75	0.77	0.81	0.78
45	0.56	0.58	0.63	0.66	0.72	0.68
60	0.42	0.44	0.51	0.55	0.62	0.57
75	0.28	0.31	0.39	0.43	0.53	0.46
90	0.13	0.17	0.27	0.32	0.44	0.36

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

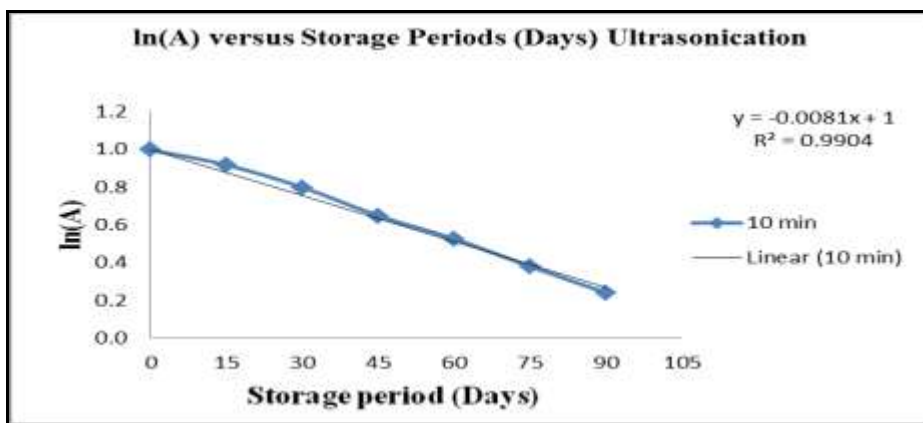


Fig. 11 Shows plot of ln(A) versus Storage Periods at 10 min

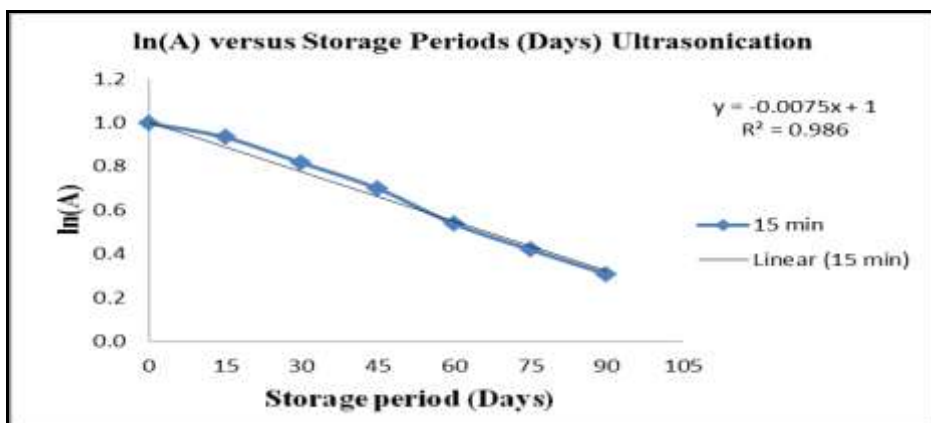


Fig. 12 Shows plot of ln(A) versus Storage Periods at 15 min

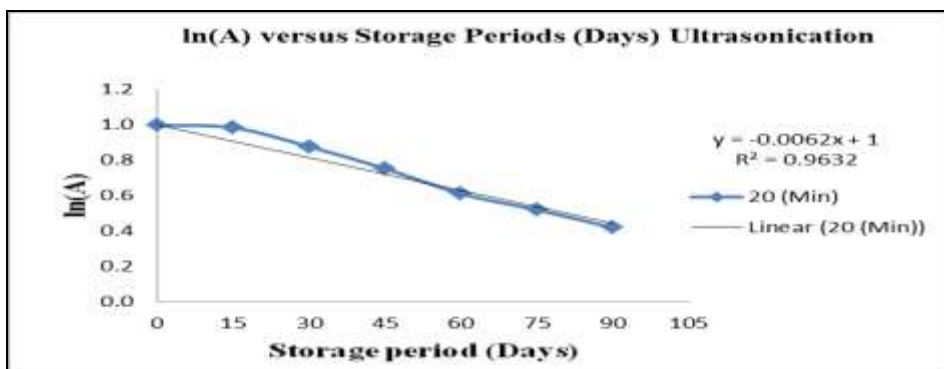


Fig. 13 Shows plot of ln(A) versus Storage Periods at 20 min

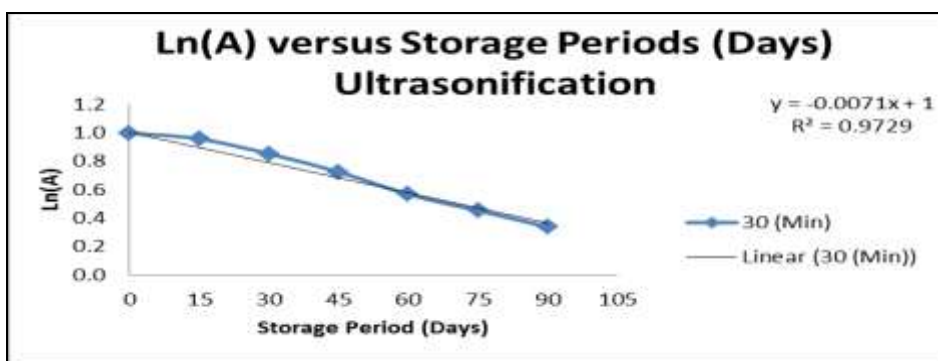
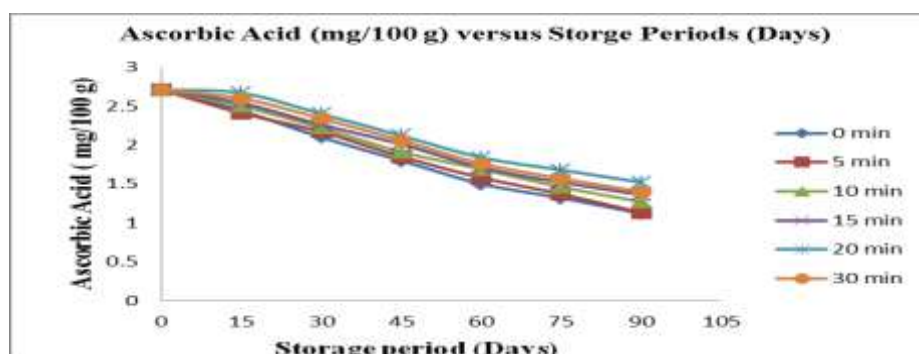
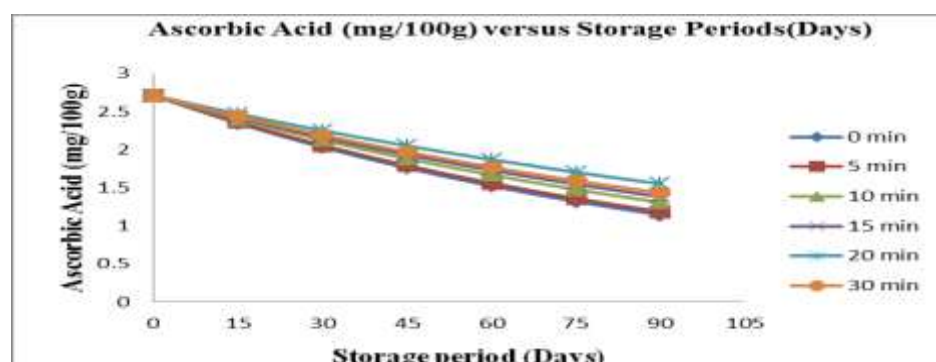


Fig. 14 Shows plot of ln(A) versus Storage Periods at 30 min

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

Time (Min)	Experimental values							Predicted values					
	Storage Period (Days)							Storage Period (Days)					
	Ascorbic acid content (mg/100 g)							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	2.41	2.17	1.85	1.58	1.37	1.13	2.36	2.06	1.79	1.56	1.36	1.18
10	2.71	2.5	2.22	1.91	1.69	1.46	1.27	2.40	2.13	1.88	1.67	1.48	1.31
15	2.71	2.54	2.26	2.01	1.71	1.52	1.36	2.42	2.16	1.93	1.73	1.54	1.38
20	2.71	2.67	2.4	2.12	1.84	1.68	1.52	2.47	2.25	2.05	1.87	1.70	1.55
30	2.71	2.6	2.34	2.06	1.76	1.57	1.4	2.44	2.19	1.97	1.77	1.59	1.43

The experimental and predicted values are shown in the table 3. There was no significant difference observed between experimental and predicted values. The experimental and predicted values of Ascorbic acid degradation during the various storage periods was shown in the figure 15 and 16. Optimized time for ultrasonication treatment of grape juice was observed 20 min and its corresponding K and R² values were 0.0062 and 0.9632.

**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 ultrasound treatment on quality of grape juice during storage period of 90 days was studied. It was concluded that ultrasonication, treatments on grape juice strongly affect the products shelf life and consumer acceptability.

It was observed that the optimum treatment for grape juice was 20 min ultrasonicated sample for 15 days and it had pH of 3.9, TSS of 17.6 °Brix, Titratable acidity of 0.84 (g/100ml), reducing sugar of 10.3 (µg/ml), ascorbic acid of 2.65 (mg/100g) microbial load of 10 (CFU/ml), and sensory score of 9. According to FDA, 5 log reductions in microbial load were attained 20 and 30 min thermosonically and aerated juice samples. The shelf life of grape juice was increased upto 15 days. The results suggest that ultrasound treatment technology could be potentially employed for the processing of grape juice and could improve its quality and

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