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Effect of Ohmic Heating on Quality and Storability of Sugarcane Juice

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A B S T R A C T

Sugarcane juice was analysed for the two different treatments such as ohmic heating and conventional heating. The study analysis showed that the total plate count decreased with severity of ohmic heating treatment which reduced from 6.3 to 3.47 log cfu/ml for 90° C and 15 min treatment. So observing the PPO inactivation, colour change and microbial reduction of the treated samples into consideration, ohmic heating of sugarcane juice at 70°C for 3 min holding time was found to be optimum. Hence, highest microbial reduction was observed in ohmic heating treatment than conventional heating treatment.

Introduction

Sugarcane (*Saccharum officinarum*) is one of the most widely relished beverages of south Asia. It is also known as noble cane, due to its high sucrose content and low fiber content is important in industrial crops of the world. Enzymatic browning is one of the major causes for deleterious changes in the sensory properties of the product thereby limiting its storage for a longer time (Bucheliand Robinson, 1994). Sugarcane juice has been used in the Ayurveda and Unani systems of medicine in India, since time immemorial. Sugarcane extract has displayed a wide range of biological effects including immune stimulation (El-Abasy *et al.*, 2002), anti-thrombosis activity, anti-inflammatory activity, vaccine adjuvant, modulation of acetylcholine release (Barocci *et al.*, 1999).

Conventional heat processing imparts the taste of jaggery and the delicate flavor of juice is adversely affected. Polyphenol oxidase is the major enzyme involved in the discoloration of sugarcane juice which can be improved by heat inactivation of enzyme. Addition of citric acid or ascorbic acid to juice also gave good pleasant dull orange colour to juice. Addition of lemon and ginger followed by pasteurization and preservation with sulphur dioxide also reduced physico-chemical changes during storage of ready-to-serve bottled sugarcane juice. However enzymatic

browning and spoilage by microorganisms due to the presence of simple sugar after extraction are responsible for its short shelf life. Ohmic heating (OH) has gained wide popularity as an alternative thermal treatment as it causes volumetric heating of the sample which leads to consistent and rapid heat generation especially in liquid foods. The rate of heat generation in OH is a function of electric field strength applied across the food material (Ramaswamy, Marcotte, Sastry, and Abdelrahim, 2014). Due to short processing times, OH causes minimum discoloration and maintains the nutritive value of the food (Leizerson and Shimoni, 2005; Wang and Sastry, 2002). This feature makes it one of the most desirable treatments particularly for sugarcane juice; as it contains sensitive flavor components that are easily destroyed at longer treatment times.

Materials and Methods

Preparation of sugarcane juice

Fully mature sugarcane stems were procured from the local market of Bhubaneswar. Fresh sugarcane was used for the extraction of sugarcane juice. Sugarcane stems were then washed by running tap water to get sugarcane free from any dust and dirt. The stems were peeled and manually cut into small pieces with the help of stainless steel knife. Sugarcane juice were extracted by motor grinder (Make: Krishna) and filtered through the sieve and muslin cloth to remove the extraneous matterandobtain a clear filtrate which was used for the study.

Application of different preservation techniques

Conventional heating

Different lots of sugarcane juices were subjected to pasteurization (at 70, 80 and 90^{o}C for 5, 10 and 15 min), A volume of 50 ml of juice was taken in a beaker and placed in lab scale water bath maintained at the desired temperature (Fig. 1). After the desired temperature of juice was achieved, it was held at that temperature for the desired time duration fresh sugarcane juice was taken as control. Three alternated replicates were conducted for each condition. The treated samples are analysed for PPO inactivation, physio-chemical, microbial and sensory attributes. All the lots of juices were filled and stored in sterilized HDPE bottle for 20 days at refrigeration temperature (4^{0}C). The samples were drawn and analyzed for physico-chemical, microbiological and sensory attributes at an interval of 5 days.

Ohmic heating

Development of ohmic heating set up

Four ohmic heating set up were developed for treatment of the sugarcane juice. Rectangular chambers were fabricated from perplex sheet with different length to obtain different electrical field strengths of 16, 32, 48 and 64 V/cm. Two stainless steel flat (100x50x5 mm) were used as electrodes and inserted in the groove located at the two ends of the rectangular chamber. The distance between the electrodes were maintained at 14.4, 7.2, 4.8 and 3.6 cm to obtain electrical field strengths of 16, 32, 48 and 64 V/cm. A power supply of 230 V and 50 Hz was used to carry out the experiment.

Ohmic heating of sugarcane juice

The ohmic heating of sugarcane juice was carried out at four different electric field strengths and the temperature rise with heating time was recorded. The best set up was used for further ohmic heating study. The sample was placed in the chamber and connected to the electrical circuit. A digital temperature

indicator was was used to record and maintain the temperature of the sugarcane juice during treatment. The samples were heated and held at 70, 80 and 90°C for 1, 2 and 3 min holding time during ohmic heating (Fig. 2).

The heating time, holding time and total processing time is given in Table 1. The samples were stored in sterilized HDPE bottle for further analysis.

Storage study

Samples processed by different treatments were packed in sterile HDPE bottle and stored under refrigerated conditions of storage for 20 days. The physico-chemical and microbial parameters of the stored sugarcane juice were conducted at 5 days storage interval for assessment of shelf life by different preservation techniques.

Determination of quality parameters

Polyphenol oxidase (PPO) enzyme assay

The assay of the enzyme was carried out as described by Ozoglu and Bayindirli (2002). One ml of 0.2 mol/L Catechol solution was added to mixture of 0.5 ml of sugarcane juice and 2 ml of phosphate buffer (pH 6.5). The absorbance was measured at 420 nm at every 1 min interval by spectrophotometer (Make: Systronics; Model: 106). The enzyme activity was estimated from the linear portion of the curve of absorbance v/s time. One unit of PPO activity was defined as 0.001A420/min.

Enzyme activity was expressed in U/mL with one unit equivalent to a variation of 0.001 absorbance per minute per mL of sample. The equation 1 was applied to calculate the enzyme activity:

$$Activity(U/ml) = \frac{(Ab_{sample} - Ab_{blank})}{0.001 \times t} \quad (1)$$

Where Ab_{sample} is the sample absorbance; Ab_{blank} is the blank absorbance; and t the incubation time of sample with reagents (min).

The activity of the samples was expressed as % Residual PPO Activity (RA) as given in Eq. (2):

$$\% RA = \frac{\text{Current Enzyme Activity}}{\text{Initial Enzyme Activity}} \times 100 \quad (2)$$

Physicochemical tests

Physico-chemical parameters such as Hunter colour value, total soluble solid (TSS), titrable acidity, reducing sugar content of treated sugarcane juice were performed as determined by AOAC International (AOAC, 2007).

TSS

The total soluble solids content of sugarcane juice (expressed as °Brix) was determined using portable digital refractometer (Make: ATAGO. Model: REF113).

Measurement of color

Colour of the sugarcane juice samples was measured by colour reader CR-20 (Konica Minolta, INC, Japan). Colorimeter was calibrated using white control sample Coordinates 'L' represented the lightness of color (0 = black; 100 = white), −a/+a greenness or redness, and −b/+b blueness or yellowness. Samples were kept in petri plates and colour value of L, a and b was measured. For each sample, three measurements were taken and averaged. The total color change (ΔE) was calculated using Eq 3 (Altan *et al.*, 2008). L_0, a_0 and b_0 are the colour values of control sample.

$$\Delta E = \sqrt{(L - Lo)^2 + (a - a0)^2 + (b - bo)^2} \quad (3)$$

Titrable acidity

The titratable acidity (expressed as % citric acid) was determined by titration with 0.1 N NaOH. It was determined by quantifying the volume of 0.01N NaOH required to raise the pH value to 8.3, and expressed as ml of 0.01 M NaOH per 10 ml of juice. About 10mlof sample was Pipette in to a 250 ml conical flask. It was added about 50ml of distilled water and few drops of phenopthalin indicator in to the conical flask. It was Titrated against to the 0.1 N NaOH up to light pink end point with solution product (Fig. 3).

$$\text{Acidity, \%} = \frac{\text{Eq. wt. of acid} \times \text{Titre value} \times \text{Normality of NaOH}}{10 \times \text{vol. of sample taken}} \quad (4)$$

Reducing sugar content

Reducing sugar content was determination by DNS (Dinitro salicylic Acid) method. About 0.5 ml of the sample was taken in a test tube and the volume was equalized to 3ml with distilled water. 3ml of DNS reagent was added to it. Then the contents in the test-tube were heated in a boiling water bath for 5mins. When the contents of the tubes were still warm, 1ml of 40% Rochelle salt solution was added to it. It was cooled and the absorbance reading was taken at 510 nm. A series of standards was prepared using glucose (0-500mg) and plot a graph. The amount of reducing sugar present in the sample was determined from the standard curve.

Microbiological tests

The sugarcane juice samples was analysed for their commercial sterility. Total Plate Count (TPC) was determined using Nutrient Agar (NA) after incubation, and for 48 h at 30^{o}C. Yeast and molds (YMC) were estimated with the help of acidified potato dextrose agar (PDA). TPC and YMC were counted in series dilution method. Results were expressed as colony forming units per milliliter.

The unit for calculation is CFU= (Number of colony ×dilution factor)/volume plated in mL

Sensory tests

Sensory evaluation of sugarcane juice processed by different treatments was carried out, using a nine-point hedonic scale, as described by Dutcosky (2013). The attributes like colour, flavour and taste were evaluated by 10 panelists and consumers. The juice was served at a temperature of about 12°C. The overall acceptability of sugarcane juice was calculated by composite scoring giving 40, 20 and 40% weightage to colour, flavour and taste score.

Statistical analysis

The experimental data were analysed by Analysis of variance (ANOVA) using MS EXCEL 2007at 5% confidence level for comparison.

Results and Discussion

Effect of different treatments on quality of sugarcane juice

Conventional heating

The effect of different processing temperature and treatment time on residual PPO activity (% RA), colour change, titrable acidiy, reducing sugar content, TSS and total plate count during conventional heating is shown in Table 2. It was observed that residual PPO activity decreased with increase in processing temperature and time ($p< 0.05$). Highest residual PPO activity was observed at 70^{0} C for 5 min, whereas it was found to be less at higher processing temperature of 90^{0}C.

However, no significant difference ($p < 0.05$) was found in the RA of the enzyme at 80^{o}C for 10 and 15 min treatment and 90^{o}C for all the time treatment suggesting the development of resistance of the enzyme to inactivation after prolonged exposure to high temperature which had also been reported by Terefe *et al.,* (2010).

Further, the change in colour was more at higher processing temperature and longer treatment time probably due to non-enzymatic browning during thermal treatment. It was observed that reducing sugar content increased significantly ($p<0.05$) with processing temperature and time. This might be due to the inversion of sugar which resulted in higher reducing sugar content leading to poor keeping quality of the juice. The TA of samples was not changing significantly and the slight increase may be attributed to some biochemical processes that might have been accelerated by the treatment. The increase in TSS was observed with heating temperature and time probably due to evaporation of water due to heat treatment. The total plate count decreased with severity of thermal treatment which reduced from 6.3 to 3.45 log cfu/ml at 90^{o}C and 15 min treatment.

So keeping PPO inactivation, colour change, reducing sugar content and microbial reduction of the treated samples into consideration, it was recommended for conventional heating of sugarcane juice at 80 ^{0}C for 10 min.

Ohmic heating

Effect of field strength on temperature rise

The temperature rise of sugarcane juice during ohmic heating at four electrical field strength is shown in Table 3. It was observed that temperature rise was slow at 16 and 32 V/cm and steady at 64 V/cm. The juice temperature attained 90^{o}C at 5, 15 and 36 min when exposed to ohmic heating at 64, 48 and 32 V/cm, respectively. Frothing of juice occurred during ohmic heating at 64 V/cm with instantaneous temperature rise and it was difficult to control. The low heating rate at 16 and 32 V/cm, resulted in prolonged treatment time which was not desirable due to quality loss. So, it was decided to conduct the ohmic heating of sugarcane juice at electrical field strength of 48 V/cm. Castro *et al.,* (2004) reported increase in enzymatic activity when exposed to low electric field strengths due to changes in the molecular spacing that accelerated the inter-chain biochemical reactions and suggested the possible use of higher electric field strength of 48 V/cm during ohmic heating.

Effect processing conditions on quality of sugarcane juice

The effect of different processing temperature and time on residual PPO activity (% RA), colour change, titrable acidiy, reducing sugar content, TSS and total plate count during ohmic heating of sugarcane juice with 48 V/cm field strength is shown in Table 4. It was observed that residual PPO activity decreased significantly ($p<0.05$) with increase in treatment temperature and processing time during ohmic heating.

The residual PPO activity was less with less colour change in ohmic heated samples treated with 70^{0} C for 3 min holding time. At higher temperature and holding time the colour change was observed to be more. The reducing sugar and titrable acidity were not changing significantly and the slight increase may be attributed to some biochemical processes that might have been accelerated by the treatment. The TSS increased with processing temperature which might be due to evaporation of water by thermal effect during ohmic heating.

Table.1 Heating time, holding time and total processing time during conventional and ohmic heating treatment

Temperature, ^{o}C	Heating time (min)	Holding time (min)	Total processing time (min)
Conventional heating			
70	10	5, 10, 15	15, 20, 25
80	12	5, 10, 15	17, 22, 27
90	14	5, 10, 15	19, 24, 29
Ohmic heating			
70	12	1, 2, 3	13, 14, 15
80	13.5	1, 2, 3	13.5, 15.5, 16.5
90	15	1, 2, 3	16,17,18

Table.2 Physico-chemical and microbial properties of sugarcane juice treated with conventional heating at different temperature and time

Temperature, ^{0}C	Holding time (min)	RA%	Colour change	Titrable acidity (g/100ml)	Reducing sugar (g/100 ml)	TSS (^{0} Brix)	TPC (log cfu/ml)
70	5	58.9±4.1	3.5±0.2	0.132±0.003	0.482±0.005	18.7±0.3	6.13±0.09
	10	55.3±3.8	3.9±0.2	0.136±0.004	0.479±0.004	19.4±0.3	5.57±0.06
	15	50.1±3.6	4.2±0.3	0.136±0.004	0.475±0.003	20.7±0.3	5.04±0.04
80	5	51.6±3.6	4.2±033	0.136±0.004	0.483±0.005	19.6±0.3	6.08±0.07
	10	**42.2**±3.2	**4.6**±0.4	**0.132**±0.003	**0.490**±0.006	**20.6**±0.4	**5.01**±0.05
	15	40.1±2.8	6.2±0.41	0.131±0.002	0.524±0.007	21.9±0.4	4.81±0.04
90	5	38.3±2.5	8.5±0.45	0.140±0.004	0.569±0.008	20.5±0.4	5.92±0.08
	10	38.0±2.2	9.1±0.43	0.136±0.005	0.572±0.009	21.4±0.5	4.34±0.04
	15	35.7±2.1	10.8±0.5	0.132±0.003	0.592±0.009	22.7±0.5	3.45±0.04
Control				0.130	0.460	18.1	6.30

Table.3 Temperature profile of sugarcane juice at different electrical field strength

Time (min)	Electric field strength, V/cm			
	16	**32**	**48**	**64**
5	27	40	41	90
10	28	44	53	
15	39	52	90	
20	41	57		
25	45	67		
30	49	75		
35	52	88		
36	54	90		
40	57			
45	59			
50	62			
55	65			
60	67			
65	70			

Table.4 Physico-chemical and microbial properties of sugarcane juice at different processing temperature and time duringohmic heating at 48 V/cm

Ohmic heating Temperature (^{0}C)	Holding time (min)	RA%	Colour change	Titrable acidity (g/100ml)	Reducing sugar (g/100 ml)	TSS (^{0} Brix)	TPC (log cfu/ml)
70	1	69.1±2.3	3.2±0.2	0.136±0.004	0.463±0.004	19.2±0.3	4.70±0.009
	2	49.42±2.2	3.6±0.2	0.132±0.002.	0.468±0.005	19.4±0.31	4.61±0.008
	3	**21.4**±2.1	**4.2**±0.3	**0.130**±0.001	**0.462**±0.004	**19.7**±0.4	4.25±0.003
80	1	42.0±2.2	6.3±0.4	0.138±0.009	0.479±0.005	19.5±0.33	4.31±0.003
	2	33.0±2.3	6.6±0.4	0.134±0.003	0.485±0.006	19.8±0.4	4.14±0.002
	3	19.7±2.1	7.0±0.4	0.130±0.001	0.487±0.006	20.1±0.5	3.96±0.002
90	1	32.2±2.3	7.6±0.5	0.134±0.003	0.500±0.007	19.4±0.32	4.12±0.003
	2	18.8±2.0	8.9±0.5	0.134±0.003	0.510±0.008	20.1±0.5	3.66±0.007
	3	16.3±2.0	10.8±0.6	0.137±0.007	0.540±0.009	20.5±0.5	3.47±0.007
Control				0.130±0.008	0.460±0.007	18.1±0.4	6.30±0.009

Table.5 Titrable acidity (g/100 ml) of sugarcane juice treated with Different methods during storage

Treatments	Storage period, days				
	0	**5**	**10**	**15**	**20**
Control	0.13±0.004	0.55±0.004	0.86±0.005	1.13±0.008	1.43±0.008
CH	0.132±0.003	0.24±0.005	0.47±0.004	0.86±0.008	1.14±0.008
OH	0.130±0.004	0.23±0.005	0.36±0.004	0.56±0.005	0.78±0.004

Table.6 Reducing sugar content (g/100 ml) of sugarcane juice during storage

Treatments	Storage period, days				
	0	**5**	**10**	**15**	**20**
Control	0.460±0.004	0.48±0.005	0.56±0.006	0.60±0.007	0.66±0.009
CH	0.490±0.005	0.50±0.007	0.52±0.003	0.54±0005	0.57±0.007
OH	0.462±0.004	0.48±0.005	0.49±0.005	0.51±0.004	0.53±0.004

Table.7 Colour change value of sugarcane during storage

Treatments	Storage period, days				
	0	**5**	**10**	**15**	**20**
Control		4.6±0.3	5.8±0.38	6.4±0.47	7.5±0.49
CH	4.6±0.2	6.5±0.33	8.9±0.39	13.4±0.46	18.2±0.5
OH	4.2±0.2	6.2±0.33	8.1±0.41	11.2±0.47	14.7±0.5

Table.8 Total plate count (log cfu/ml) of sugarcane juice during storage

Treatments	Storage period, days				
	0	**5**	**10**	**15**	**20**
Control	6.30±0.06	6.32±0.07	6.56±0.079	6.98±0.08	7.23±0.088
CH	5.01±0.065	5.23±0.076	5.60±0.079	5.81±0.082	5.98±0.089
OH	4.25±0.064	4.45±0.077	4.80±0.078	5.01±0.084	5.11±0.089

Fig.1 Conventional heating of sugarcane juice in water bath

Fig.2 Ohmic heating set up

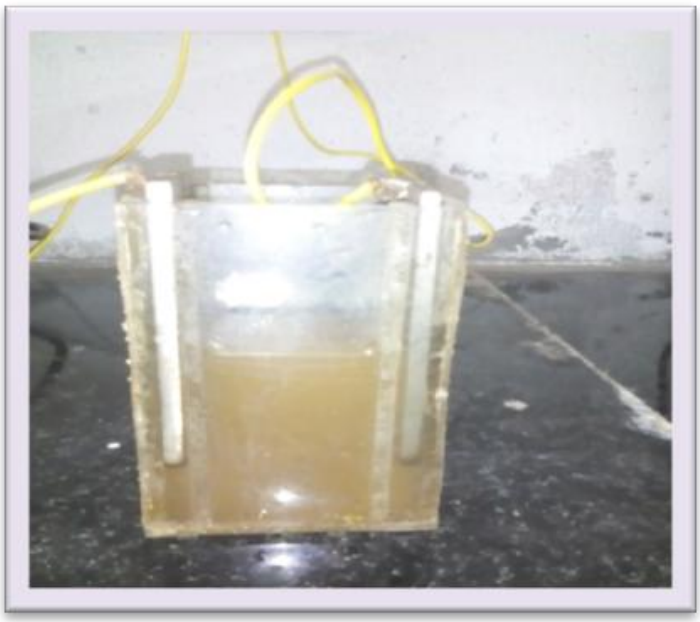


Fig.3 Change in titrable acidity of sugarcane juice processed

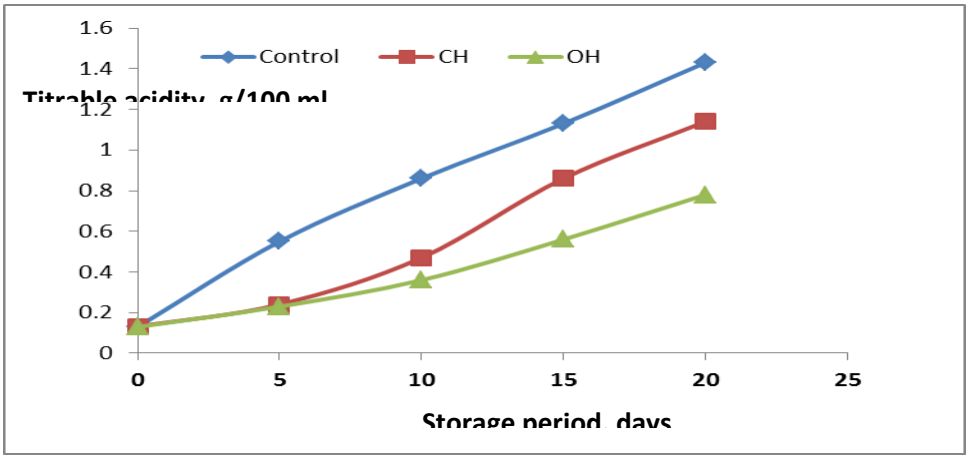


Fig.4 Change in reducing sugar of sugarcane juice processed by different treatments during storage

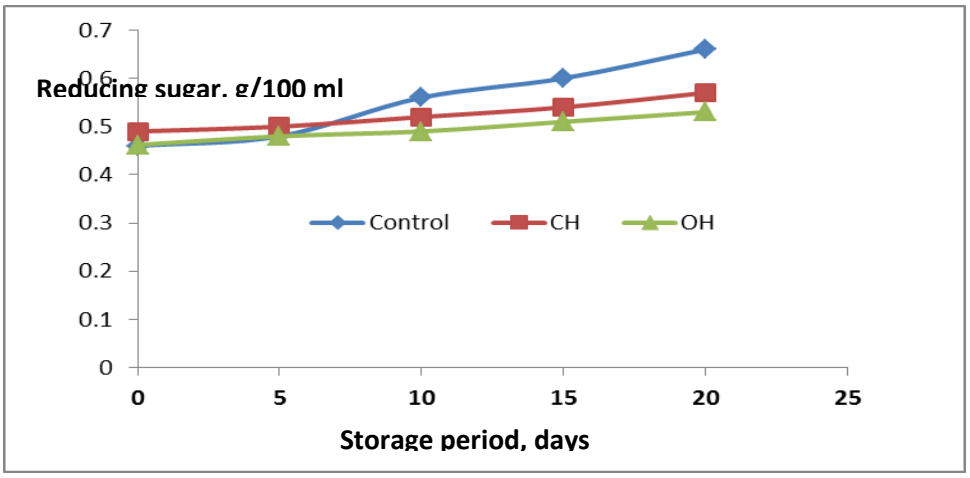


Fig.5 Change in Change in colour of sugarcane juice processed by different treatments during storage

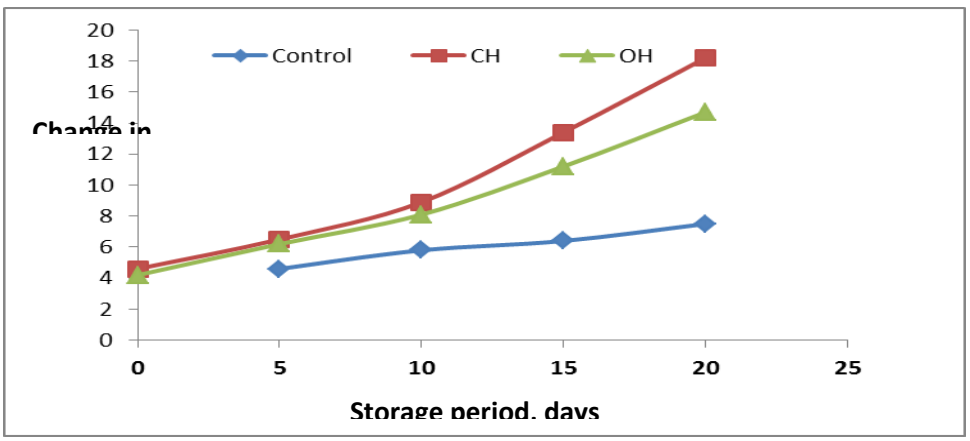
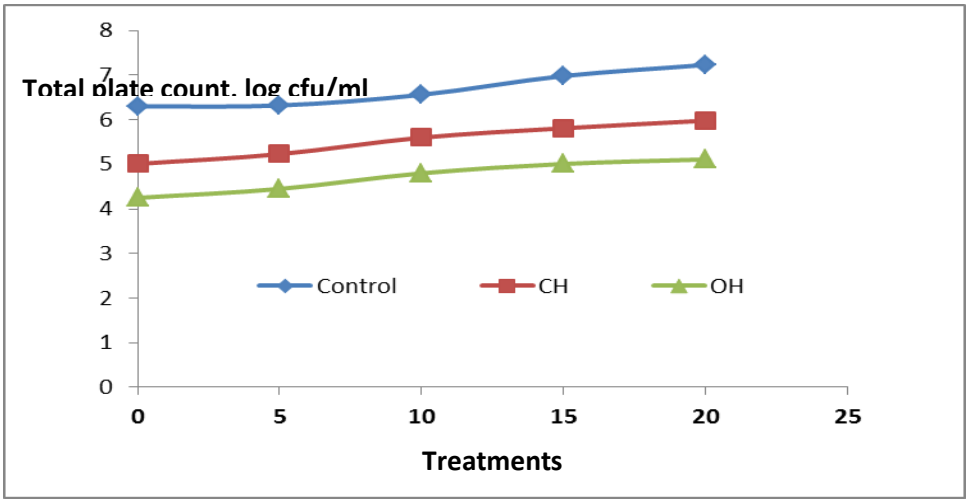


Fig.6 Change in microbial load of sugarcane juice processed during storage



The total plate count decreased with severity of ohmic heating treatment which reduced from 6.3 to 3.47 log cfu/ml for 90^{o}C and 15 min treatment. Saxena *et al.,* (2016) reported that higher field strength of 48 V/cm resulted in a significant reduction in % RA and higher degree of microbial reduction probably due to the combined effect of heat as well as electric current.

So keeping PPO inactivation, colour change and microbial reduction of the treated samples into consideration, ohmic heating of sugarcane juice at 70 ^{0}C for 3 min holding time was found to be optimum.

Storage study

The sugarcane juice treated with different methods at optimum dose was stored in sterile HDPE bottle under refrigerated condition. The quality parameters such as titrable acidity, reducing sugar, colour change and microbial load of the samples were determined after 5 days interval.

Titrable acidity

The TA of sugarcane juice increased significantly ($p < 0.05$) with storage period. The increase in TA of ohmic heated sample from 0.13 to 0.78 g/100 ml was less compared to conventional heating treatment indicating better storability (Table 5). The acidity of ohmic heated juice increased to 0.37 after 10 days of storage under ambient condition with acceptable odour.

Reducing sugar

The RS content for untreated juice increased significantly ($p < 0.01$) from 0.46 to 0.66 after 20 days of storage (Table 6 and Fig. 4). The increase was less in ohmic heated samples. Increase in reducing sugar during storage of sugarcane juice was also reported by Saxena *et al.,* (2016). The increase in reducing sugar during storage was probably due to the action of dextransucrase on sucrose releasing RS molecule.

Change in colour

The change in colour during storage of sugarcane juice treated with different processing conditions is given in Table 7. The colour change was found to be more in ohmic heating samples as compared to conventionally heated samples (Fig. 5). The higher colour change in ohmic heated samples

was probably due to non-enzymatic browning and formation of viscous jelly like substance called dextran by the action of enzyme.

Microbial load

The microbiology parameters were investigated to observe the quality ohmic heated and conventionaly heated sugarcane juice samples. Total plate count (TPC) value increased significantly ($p<0.05$) with storage period. The increase was highest in control sample and lowest in ohmic heated sample (Fig. 6). The microbial count in control sample increased from 6.3±0.06 to 7.23±0.088 after 20 days of storage under refrigerated storage (Table 8). Ohmic heated sample could be stored up to 10 days having TPC value less than 10^5.The mechanism of microbial destruction by heat is well known and higher degree of inactivation by OH treatment was due to the combined effect of heat as well as electric current. Microbial inactivation by electric field has been reported to be majorly by electroporation (Tsong, 1991) but some researchers have also suggested the formation of microbicidal agents such as chlorine, hydrogen peroxide etc., due to electric discharge in liquid media, that alter the DNA and cytoplasmic activity of the cells (Hulsheger, Potel, and Niemann, 1981).

Highest residual PPO activity was observed at 70^0 C for 5 min, whereas it was found to be less at higher processing temperature of 90^0C. The change in colour was more at higher processing temperature and longer treatment time. Heating sugarcane juice to 80 ^{0}C and holding for 10 min was found to be the optimum condition in conventional heating. Frothing of juice occurred during ohmic heating at 64 V/cm with instantaneous temperature and low heating rate at 16 and 32 V/cm, resulted in prolonged treatment time. Electrical field strength of 48 V/cm was found to be suitable for ohmic heating of sugarcane juice. PPO activity decreased significantly with increase in treatment temperature and processing time during ohmic heating. The reducing sugar and titrable acidity were not changing significantly during ohmic heating. At higher temperature and holding time the colour change was observed to be more. Ohmic heating of sugarcane juice at 70 ^{0}C for 3 min holding time was found to be optimum. From the above study, it could be concluded that ohmic heating of sugarcane juice at 70^0C for 3 min holding time with 48 V/cm electrical field strength resulted in higher PPO inactivation and microbial reduction which could be stored safely up to 10 days under refrigerated storage condition with some compromise in the colour of the juice.

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Despite the problems of electrochemical degradation associated, OH has been successfully studied for its use in preheating, blanching, and extraction (Lakkakula, Lima, and Walker, 2004; Leizerson and Shimoni, 2005.)

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