High pressure, temperature and time-dependent effects on enzymatic and microbial properties of fresh sugarcane juice

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Abstract Efficacy of variable high pressure, temperature and time on the browning causing enzymes and microbial activities, which are major spoilage factors during preservation of sugarcane juice, was studied. The juice was processed at 200–600 MPa pressure for 2–8 min at 40 and 60 °C and their effect on polyphenol oxidase and peroxidase as well as microbiological quality in terms of total plate count, yeast and molds and total coliforms was studied. Application of high pressures were found to cause significant decrease in enzymatic and microbial activities. The effects were found to be significantly more pronounced at 60 °C as compared to 40 °C. Process time also caused significant ($p < 0.05$) negative effect on microbial and enzyme activities. The sugarcane juice treated at 600 MPa for 6 min at 60 °C was found sufficient to inactivate the microbial counts completely. Whereas, enzymes were found to be completely inactivated in the samples processed at 600 MPa for 8 min at 60 °C. A pressure of 600 MPa at 60 °C for 8 min could be applied during commercial preservation of sugarcane juice for getting complete inactivation of browning causing enzymes and spoilage causing microorganisms.

Keywords Sugarcane juice · High pressure · Polyphenol oxidase · Peroxidase · Microbiology

Introduction

Sugarcane (*Saccharum officinarum*) juice is relished as refreshing beverage throughout India as well as in other countries. It is prepared from fresh mature sugarcane by crushing and extracting the juice. India is second largest producer of sugarcane after Brazil, producing around 352.16 MT of sugarcane in the year 2015–16 (Agriculture Statistics at a Glance 2016). Bagasse left over after juice extraction is also being utilized in several means. A small portion of sugarcane is also utilized for beverage preparation which is highly liked throughout the country. Sugarcane juice contains approximately 15% natural sugar and it is also rich in vitamins and minerals making it a healthier beverage (Qudsieh et al. 2001). Sugarcane juice is also known for its medicinal and therapeutic properties. Sugarcane juice is considered good for preventing and healing sore throat, cold, flu and jaundice. A major problem in sugarcane juice is that it turns blackish during storage due to the enzymatic activities (Chauhan et al. 2002). Another major problem is sedimentation which appears during storage. Conventional heat processing do inactivate browning enzymes but also causes development of jaggery like flavor impeding the delicate flavor of the juice and making it unacceptable (Sivasubramanian and Pal 1994). Irradiation treatment in combination with chemical additives has also been tried for preservation of sugarcane juice (Mishra et al. 2011). Sugarcane juice also spoils fast due to presence of high amount of sugars which gets fermented by microorganisms such as *Leuconostoc* sp. (Khare et al. 2012). High pressure processing is a relatively new technology for preservation of food items. It is a most promising non-thermal technology being used worldwide for food preservation including fruit and vegetable juices. Application of high pressures has been found to decrease

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the enzymatic and microbial activities in different fruit and vegetable juices (Unni et al. 2011). As enzymatic browning and microbial spoilage are the major problems in sugarcane juice, therefore, the present work was carried out to study the effect of high pressures at variable temperatures and time durations on enzymatic and microbial quality of sugarcane juice, so that this technology can be explored for preservation of sugarcane juice, which is otherwise a problematic area in thermal processing.

Materials and methods

Sugarcane (*Saccharum officinarum* Var. CO419) juice were procured from local market of Mysore, India just after juice extraction and brought to Defence Food Research Laboratory, Mysore in an ice box. The juice was processed at 200, 400 and 600 MPa pressures for 2, 4, 6 and 8 min at a temperature of 40 and 60 °C (Table 1). A laboratory scale high pressure food processing system (ISO-LAB

FPG9400, Stansted Fluid Power Ltd., Stansted, UK) consisting of a high pressure vessel (2 l capacity) with dual high pressure pumps and pressure intensifiers, was used to achieve and maintain the desired pressure in the pressure vessel. The high pressure vessel was surrounded by a liquid circulating jacket connected to a heating–cooling system for maintaining the exact processing temperature. The pressure transmitting fluid used was 30% mono-propylene-glycol (supplied by M/S Hydraulic Systems, Ahmedabad, India). The ramp rates for pressurization and decompression were set at 600 and 2000 MPa/min, respectively. The initial temperature increase during pressure build-up (about 2–3 °C/100 MPa) was taken into consideration in order to achieve the desired operating temperature during pressurization. Pressure, temperature and time were constantly monitored and recorded (at 1 s intervals) during the process using a SCADA based software (Stansted Fluid Power Ltd., Stansted, UK).

The fresh and high pressure processed juice was analyzed for enzymatic (poly phenol oxidase and peroxidase)

Table 1 Effect of pressure, temperature and time on enzymatic and microbial activities of sugarcane juice

Pressure (MPa)	Temperature (°C)	Time (min)	PPO (units/ml/min)	POD (units/ml/min)	Total plate count (cfu/ml)	Coliform counts (cfu/ml)	Yeast and mold count (cfu/ml)
Fresh	–	–	350 ^a	4150 ^e	9×10^4	3×10^2	8×10^2
200	40	2	320 ^b	3900 ^f	4×10^4	ND	3×10^2
		4	135 ^h	4200 ^d	6×10^3	ND	1×10^2
		6	160 ^c	4450 ^c	9×10^2	ND	ND
		8	160 ^c	4550 ^b	8×10^2	ND	ND
		2	155 ^d	4750 ^a	8×10^2	ND	ND
	60	4	160 ^c	3800 ^h	7×10^2	ND	1×10^2
		6	155 ^d	3550 ^k	1×10^3	ND	ND
		8	150 ^e	3700 ^j	4×10^2	ND	ND
	40	2	145 ^f	3750 ⁱ	8×10^2	ND	ND
		4	145 ^f	3850 ^g	7×10^2	ND	ND
		6	150 ^e	3050 ^m	7×10^2	ND	ND
		8	155 ^d	3200 ^l	4×10^2	ND	ND
400	60	2	145 ^f	2650 ⁿ	1×10^2	ND	1×10^1
		4	150 ^e	2100 ^q	1×10^2	ND	ND
		6	140 ^g	2150 ^p	2×10^2	ND	ND
		8	145 ^f	1875 ^t	9×10^2	ND	ND
		2	155 ^d	2450 ^o	1×10^2	ND	2×10^2
	40	4	160 ^c	2000 ^r	1×10^2	ND	1×10^2
		6	145 ^f	2100 ^q	1×10^2	ND	ND
		8	135 ^h	1900 ^s	7×10^1	ND	ND
	60	2	160 ^c	1500 ^u	8×10^2	ND	ND
		4	140 ^g	1050 ^v	6×10^2	ND	ND
		6	110 ⁱ	380 ^w	ND	ND	ND
		8	ND	ND	ND	ND	ND

Values with different superscripts in the same column differ significantly ($p < 0.05$)

ND Not detected

activities as per the procedure described by Gonzalez et al. (2000). Microbiological analyses of the juice were carried out for total plate counts, yeast and mould counts and total coliform counts according to APHA (1992) procedures. The pH was determined using a microprocessor based pH meter (Century, Model CP931, Bangalore, India). Total soluble solids (TSS) were measured using a hand refractometer (Erma, Tokyo, Japan). All measurements were performed in triplicates for different physico-chemical parameters and the data obtained were analyzed statistically for analysis of variance by one way ANOVA with least significant difference (LSD) at $p < 0.05$ using Statistica 7 software (Stat Soft, Tulsa, OK, USA).

Results and discussion

Fresh sugarcane juice had a pH and total soluble solids (TSS) values of 4.72 and 9.2°Brix. High pressure processing caused a non-significant change in the pH and TSS content of the sugarcane juice (data not shown). High pressure has been found in earlier reports also to cause non-significant changes in the pH and TSS of fruit and vegetable juices (Chen et al. 2015). The polyphenol oxidase (PPO) and peroxidase (POD) enzymes are mainly responsible for enzymatic browning reactions leading to change in the color of the fresh fruit and vegetable juices. Sugarcane juice contains significant amount of PPO and POD activities, which need to be checked immediately after juice extraction to prevent browning of juice. The PPO and POD activities in fresh sugarcane juice were found to be 350 and 4150 absorbance units/ml/min, respectively (Table 1). Bucheli and Robinson (1994) studied contribution of enzymatic browning to colour in sugarcane juice and found POD to be more heat stable as compared to PPO in sugarcane juice, however, PPO contributed more towards enzymatic browning. In the present study, application of high pressures caused significant ($p < 0.05$) decrease in the PPO and POD activities. High pressure processing at 200, 400 and 600 MPa for 2–8 min at 40 and 60 °C significantly ($p < 0.05$) reduced the enzymatic activities. The PPO activity was found to decrease continuously with increase in pressure from 200 to 600 MPa, whereas, an increase in POD activity was observed at lower pressures (i.e. 200) which decreased further with increase in the pressure to 400 and 600 MPa. PPO activity showed more uniform decreasing trend as compared to POD activity with increase in pressure, temperature and time. The effect of temperature was found to cause significantly ($p < 0.05$) higher enzyme inactivation as compared to pressure and processing time. Application of high pressure for longer durations also caused significant ($p < 0.05$) decrease in enzymatic activities. PPO and POD activities

were completely inactivated in the juice processed at 600 MPa for 8 min at 60 °C. Thus, it is clear that enzymatic activities in fresh sugarcane juice can be inactivated at higher pressures (i.e. 600 MPa) and temperatures (i.e. 60 °C) processed for sufficient time period (i.e. 8 min). Application of high pressures caused inactivation of PPO activity to a greater extent as compared to the POD activity, however, at higher pressures, i.e. 600 MPa, activity of both the enzymes were completely inactivated. Various fruit and vegetable juices showed similar trends when processed at higher pressures. Juarez-Enriquez et al. (2015) reported decrease in PPO activities in apple juice processed at 430 MPa for 7 min. Chakraborty et al. (2015) also reported reduction in PPO and POD activities in high pressure processed pineapple puree. Regeneration of enzymatic activities has also been observed in high pressure processed juices after processing, therefore, it is recommended that enzymatic activities need to be checked during storage of the juices. Enzymes are basically protein in nature which gets unfolded during pressurization due to rupture of covalent bonds. These unfolded strands may rejoin again by bond formations during storage (Chauhan et al. 2013). Phenolic contents present in juice also contribute to the colour change of sugarcane juice.

The effect of high pressure processing on total plate counts, yeast and mold counts and total coliform counts is given in Table 1. The initial total plate, yeast and mold and coliform counts were found to be 9×10^4 , 8×10^2 and 3×10^2 cfu/ml, respectively. Application of high pressures at 200, 400 and 600 MPa decreased all the three types of microbial counts significantly ($p < 0.05$); maximum reduction being at higher pressures showing pressure sensitivity of the microorganisms. Coliforms were found to be more sensitive towards pressure followed by yeast and molds and total bacterial counts. Application of 200 MPa for 2 min caused complete inactivation of coliforms, whereas, application of 200 MPa pressure for 6 min inactivated yeasts and moulds completely. Total plate count decreased gradually with increase in pressure from 200 to 600 MPa and were completely inactivated at a pressure of 600 MPa, at 60 °C in 6 min. Process temperature and process time also had significant ($p < 0.05$) effect on microbial inactivation. All the three variables, i.e. pressure, temperature and time, had shown positive effect towards microbial inactivation. Thus, it is possible to maintain the microbial safety and quality of sugarcane juice during storage by application of high pressures. Several studies exist in literature indicating inactivation of microorganisms by high pressure processing in different food products including fruit and vegetable juices. Cao et al. (2012) pressure processed strawberry juice at 600 MPa for 4 min for microbial inactivation and the juice stayed for 6 months at 4 °C. Patterson et al. (2012) treated carrot juice at 500

and 600 MPa pressures for 1 min at 20 °C and found that high pressure treatment reduced the total counts approximately by 4 log cfu/ml and there was very little growth of the survivors during 4 °C storage for up to 22 days. These studies reflects efficacy of high pressures towards microbial inactivation and as such this technology has shown promise towards preservation of sugarcane juice and in maintaining the microbiological quality and safety of the juice.

Conclusion

High pressure processing had shown promise towards preservation of sugarcane juice. Application of high pressures caused significant reduction of browning causing enzymes, i.e., PPO and POD activities besides ensuring microbial safety. Total plate count, yeast and mould count as well as coliform counts were completely inactivated in sugarcane juice processed at 600 MPa pressure and at a temperature of 60 °C for 6 min. However, enzymes were completely inactivated in sugarcane juice samples at 600 MPa pressure and at a temperature of 60 °C in 8 min. The enzymatic as well as microbial activities were found to be pressure, temperature and time dependent in sugarcane juice. Further work is needed with regards to see the storage stability and quality of the high pressure processed juices in terms of physico-chemical, enzymatic and microbial changes occurring during ambient or low temperature storage. As such, high pressure processing has potential for preservation of temperature sensitive sugarcane juice without causing flavor changes.

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