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Effect of High Pressure Processing on Enzyme Inactivation and Microbial Destruction of Sugarcane Juice

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ABSTRACT

Keywords

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The shelf-life of fresh sugarcane juice is limited due to enzymatic browning and microbial growth. Browning is caused by polyphenol oxidase (PPO) enzyme and the microbial spoilage occurs mainly due to yeasts and moulds, aerobic bacteria and coliforms. High pressure processing is one of the novel food preservation methods used as an alternative to thermal treatment. The impact of high pressure processing (HPP) conditions on physicochemical properties (colour, TSS, pH), PPO activity and microbial population of fresh sugarcane juice was studied. The samples were pressurized at 300 and 400 MPa for 10 to 25 minutes dwell time at ambient temperature (25 ± 2 °C). At 300 MPa, the maximum of 3.3, 3.84 and 6.30 \log_{10} cycles reduction was observed in total bacterial count, yeast & mould and coliforms respectively; whereas the numerals for those at 400 MPa were 4.44, 5.31 & 6.30 \log_{10} cycles, respectively. The inactivation of PPO in sugarcane juice increased with increase in either pressure or dwell time. Maximum of 57% inactivation of PPO was obtained under 400 MPa pressure at 25 min. No significant changes ($p > 0.05$) were observed in pH and °Brix and also the colour changes were not visually noticed ($\Delta E < 2.5$) for pressurized juice at different dwell times.

Introduction

Fresh sugarcane juice, a refreshing healthy drink, is a popular beverage relished by the consumers in many countries especially during the summer months. It is a cheap and high energy drink which is sweet, healthy and alternative to aerated drinks. Sugarcane juice is a nutritional product containing natural sugars, minerals and organic acids and has good medicinal properties. Being a health promoting food, it strengthens the stomach, kidneys, heart, eyes, brain and sex organs (Karthikeyan, *et al.*, 2010).

As sugarcane juice is rich in sugars, it starts fermenting immediately after extraction and turns to brown due to polyphenol oxidase activity (Qudsieh *et al.*, 2002). Besides endogenous enzyme activity, quality degradation due to microbial spoilage is also a factor of concern. Microbial fermentation of juice turns its taste to sour within hours of extraction (Yusof *et al.*, 2000). These negative effects limit the preservation and marketing of raw sugarcane juice. Hence, development of a preservation method, which

can control microbial growth in raw sugarcane and maintains its freshness, is a challenging problem.

Thermal processing is commonly used by the food industry for enzyme and microbial inactivation and is generally effective in this regard. However, thermal processing may cause undesirable changes in product's sensory as well as nutritional attributes, in specific to sugarcane juice it imparts jaggery flavour, which is undesirable for the consumer (Chauhan *et al.*, 2002). Over the last 20 years, there has been a great deal of interest shown by both the food industry and academia in exploring alternative food processing technologies that use minimal heat and/or preservatives. One of the technologies that have been investigated in this context is high-pressure processing (HPP). HPP, otherwise known as high-hydrostatic pressure (HHP) and ultra-high-pressure processing (UHP), is a novel non-thermal technology that subjects juice products, or other foodstuffs, already sealed in their final packaging to high-pressure. HHP has been found to inactivate microorganisms and denature several enzymes without greatly affecting nutritional qualities. It has the potential to produce quality foods which are microbiologically safe with an extended shelf-life. Application of High pressure processing on enzyme inactivation and microbial destruction for shelf life extension of fresh sugarcane juice was the principle objective of the current research.

Materials and Methods

Preparation of sugarcane juice samples

The Sugarcane variety 93A145 was selected for the study and the canes obtained from the Agricultural farm of Indian Institute of Technology were manually peeled, washed with fresh water and crushed immediately

with laboratory model stainless steel sugarcane crusher (make: SGK Industries, Hyderabad). The juice (50 ml) was then filtered for clarification using 3-layered muslin cloth and packed in low-density polyethylene (LDPE, 80±2 µm) films for pressure treatments.

HP treatment

Packed sugarcane juice samples were immediately pressurized at 300 and 400 MPa for a dwell time of 10, 15, 20, 25 min in a batch mode HPP system (Model: S-I L-100-250-09-W; Stansted Fluid Power Systems, U.K) having 2-L capacity cylindrical vessel with 100 mm inner diameter and 250 mm depth. The temperature of the immersion medium was sensed by a thermocouple and change in temperature of the immersion medium during pressurization was recorded using a programmable logic controller (PLC). All the samples treated at different pressure levels and dwell time were analysed for quality and compared with untreated juice samples.

Microbial analysis

The microbial load of the untreated and treated juice samples was determined using spread plate method. To prepare the dilutions, each of the duplicate treated pouches was opened under aseptic conditions and 1 ml of juice was taken from each pouch, mixed with 9 ml of sterile 0.1% (w/v) peptone water. The detection limit of the analysis was 10 cfu/ml. The total bacterial count (TBC) was determined after incubating at 37 °C for 18-24 h. The Yeast and mould count (YMC) was determined using potato dextrose agar (PDA) after incubation at 27°C for 36 h. The coliform count was determined using violet red bile agar (VRBA) and the plates were incubated at 37 °C for 18-24 h. After incubation, measurement was taken as colony

forming units (cfu/ml). Analysis was done in triplicates and the mean value of log₁₀ cfu/ml of the sample was reported.

Measurement of enzyme activity

Enzyme extraction and assay

Analytical grade chemicals, namely, Catechol, polyvinylpyrrolidone (PVP) were used and PPO extraction was done for assay in 0.05 M phosphate buffer of pH 7.0. Processed sugarcane juice of 1.5 ml was mixed with 0.5 ml of 0.05 M phosphate buffer of pH 7.0 and 0.1 g PVP in a 2 ml micro tube and centrifuged at 10000 rpm for 20 minutes at 4 °C. The supernatant containing enzymes was taken out and PPO activity was assessed spectrophotometrically at 420 nm, by recording the absorbance increase for 5 minutes. The sample cuvette contained 1.25 ml of 0.1 M catechol with 1.55 ml of 0.05 M phosphate buffer (pH 7) and 0.2 ml sugarcane juice. The blank was prepared with 1.25 ml of catechol and 1.75 ml of phosphate buffer. Enzyme activity was calculated from the linear portion of plot of absorbance against time and expressed as $\Delta\text{Abs} \cdot \text{min}^{-1} \cdot (\text{ml sample})^{-1}$. The relative activity (%) of enzyme was expressed as A/A_i , where A_i represents the initial activity of the enzyme in untreated sample.

Physico-chemical properties

Colour measurement

CIE colour parameter L*(lightness), a* (red-green) and b* (yellow-blue) of the samples was estimated using portable colorimeter (Model: Spectro-guide 45/0 gloss; BYK Gardner, Germany). From the values of L*, a*, b*, total colour difference ΔE^* was calculated. ΔE^* is a parameter that quantifies the overall colour difference of a given sample to a reference sample. It was calculated using the following equation:

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0.5}$$

Where $\Delta L^* = (L_1^* - L_0^*)$, $\Delta a^* = (a_1^* - a_0^*)$, $\Delta b^* = (b_1^* - b_0^*)$. Subscript '0' depicts the colour value for reference sample and subscript '1' depicts the colour value for the sample being analyzed.

Total Soluble Solid (TSS) and pH measurement

Total soluble solids (TSS) of the juice sample was determined using a digital hand refractometer (model: PAL-1; make; Atago, Japan). The pH of the sample was determined using a pH meter (model: 877, titrino plus, make Metrohm). pH indicator probe was inserted into the sample at 25 °C and stable reading was taken as pH value.

Statistical analysis

Significance test viz. Fisher's least significance difference (LSD) and Tukey's HSD test were carried out at 95% confidence interval for all the attributes in SPSS software version 2.0 (IBM SPSS Statistics, USA). Differences at $p < 0.05$ were considered to be significant.

Results and Discussion

PPO inactivation

Polyphenol oxidase is one of the major oxidative enzyme involved in browning reactions, affecting the colour and flavour of food products (Zabetakis *et al.*, 2000). Changing to brown colour affects the appearance and organoleptic properties of the products. Hence, inactivation of PPO enzyme is highly desirable. Inactivation per cent of polyphenol oxidase of sugarcane juice treated at 300 MPa and 400 MPa pressures at ambient temperature for dwell time of 10, 15, 20 and 25 min are summarized in table 1. It was observed that the percentage of PPO

inactivity increased significantly with increasing pressure and time from 10 to 25 min. Keeping the pressure same and increasing the dwell time, the rate of inactivation increased significantly.

The polyphenol oxidase inactivity in sugarcane juice after treatment at 300 MPa at ambient temperature ranged from 37.0 to 57.0 % (Table 1). Also, with increase in pressure from 300 to 400MPa, significant increase in PPO inactivation was observed. A maximum inactivation of 57% was observed in juice sample treated with 400 MPa. As reported by Chakroborty *et al.*, (2012), PPO inactivation was 50% in strawberry puree under high pressure processing at 600 MPa/80 °C/10min. While in Apricot, strawberry and grape PPO inactivation could be achieved at

pressures exceeding 100, 400 and 600 MPa respectively (Hendrickx *et al.*, 1998). Depending upon pH, pressure of 100-700 MPa was needed for inactivation of apple PPO (Anese *et al.*, 1994). Enzyme inactivation under high pressure domain is the pressure induced irreversible structural rearrangement from native to denatured state finally affecting its molar volume (Balny and Masson 1993; Gross and Jaenicke 1994).

Effect on microorganisms

The level of inactivation of pathogens in food deemed necessary to achieve an adequate margin of safety varies depending on the severity of the hazard posed by the organism, and whether subsequent handling and storage conditions are likely to increase the risk.

Table.1 PPO inactivation (%) at 300 and 400 MPa at different dwell times

Dwell time (min)	Residual activity (%)		PPO inactivation (%) at	
	300 MPa	400 MPa	300 MPa (%)	400 MPa (%)
10	63±1.1	59±2.2	37±1.1 ^{bA}	41±2.2 ^{bB}
15	58±2.0	54±2.1	42±2.0 ^{cA}	46±2.1 ^{cB}
20	53±2.2	48±2.5	47±2.2 ^{dA}	52±2.5 ^{dB}
25	49±2.0	43±2.0	51±2.0 ^{eA}	57±2.0 ^{eB}
Control	100	100	0.0±0.0 ^a	0.0±0.0 ^a

Values are presented as mean± standard error (N=6)

Alphabets in small letters (a, b, and c) and capital letters(A and B) in the superscripts denote that the mean values are statistically different within the column and row respectively at p <0.05.

Table.2 Total bacterial count (log cfu/ml) at 300 and 400 MPa at different dwell times

Dwell time (min)	Total bacterial count (log ₁₀ cfu/ml) at		Reduction in Total bacterial count (log cycles)	
	300 MPa	400 MPa	300 MPa	400 MPa
10	4.78±0.31 ^{dA}	4.14±0.22 ^{dA}	1.77	2.41
15	4.28±0.22 ^{cB}	3.48±0.16 ^{cA}	2.27	3.07
20	3.80±0.21 ^{bB}	2.78±0.13 ^{bA}	2.75	3.77
25	3.25±0.15 ^{aB}	2.11±0.11 ^{aA}	3.30	4.44
Control	6.55±0.11 ^e	6.55±0.11 ^e		

Values are presented as mean± standard error (N=6)

Alphabets in small letters (a, b, and c) and capital letters (A and B) in the superscripts denote that the mean values are statistically different within the column and row respectively at p <0.05.

Table.3 Yeast and mould count (log₁₀cfu/ml) and Coliforms count (log₁₀cfu/ml) at 300 and 400 MPa at different dwell times

Dwell time (min)	Yeast and mould count (log ₁₀ cfu/ml)		Reduction in Yeast & mould (log cycles)		Coliforms count (log ₁₀ cfu/ml)	
	300 MPa	400 MPa	300MPa	400 MPa	300 MPa	400 MPa
10	3.81±0.2 ^{dB}	3.15±0.15 ^{dA}	1.98	2.64	ND	ND
15	3.20±0.25 ^{cB}	2.28±0.18 ^{cA}	2.59	3.51	ND	ND
20	2.59±0.12 ^{bB}	1.4±0.11 ^{bA}	3.20	4.39	ND	ND
25	1.95±0.05 ^{aB}	0.48±0 ^{aA}	3.84	5.31	ND	ND
Control	5.79±0.21 ^c	5.79±0.21 ^c			6.3±0.21	6.3±0.21

Values are presented as mean± standard error (N=6)

Alphabets in small letters (a, b, and c) and capital letters (A and B) in the superscripts denote that the mean values are statistically different within the column and row respectively p <0.05.

ND: not detected

Table.4 TSS and pH of sugarcane juice processed at 300 and 400MPa at different dwell times

Pressure (MPa)	Dwell time (min)	TSS (°B)	pH
300	10	19.42± 0.938	5.10± 0.063
300	15	19.55± 0.938	5.10± 0.063
300	20	19.55± 0.938	5.15± 0.063
300	25	19.50± 0.938	5.10± 0.063
400	10	21.42± 0.938	5.18± 0.063
400	15	21.32± 0.938	5.24± 0.063
400	20	21.30± 0.938	5.22± 0.063
400	25	21.25± 0.938	5.24± 0.063
Control		19.55± 0.938	5.11± 0.063

Table.5 Change in colour values (ΔE) at 300 and 400 MPa at different dwell times

Dwell time (min)	Change in colour values (ΔE) at	
	300 MPa	400 MPa
10	0.25±0.02 ^{aA}	0.85±0.07 ^{aB}
15	0.67±0.05 ^{bA}	0.88±0.06 ^{aB}
20	0.85±0.06 ^{cA}	1.31±0.08 ^{bB}
25	1.22±0.09 ^{dA}	1.67±0.07 ^{cB}

Values are presented as mean± standard error (N=6)

Alphabets in small letters (a, b, and c) and capital letters (A and B) in the superscripts denote that the mean values are statistically different within the column and row respectively p <0.05.

In addition to microbiological inactivation, the economic feasibility of HHP processing requires that treatment conditions be optimized to achieve the lowest pressure/shortest time combinations needed to eliminate pathogens of concern from the food to be treated. Treated and untreated sugarcane juice samples were analyzed for Total bacterial plate count (TPC), coliforms and yeast and mould count (Y&M) and are presented in tables 2 and 3.

Both intensity and duration of pressurization reduced total viable counts of microbes immediately after HP treatments. It was observed that the increase in dwell time from 10 to 25 min within same the pressure level, the total bacterial count and yeast and mould count decreased significantly. Also with an increase of pressure from 300 to 400 MPa, the total bacterial count and Yeast and mould count reduced significantly. At 300 MPa, the maximum of 3.30 and 3.84 \log_{10} reduction was observed in total bacterial count, yeast & mould respectively; whereas the numerals for those at 400 MPa were 4.44 and 5.31 respectively.

It was reported by Barros *et al.*, (2005) that more than 4 log cycle reduction was attained in Yeast & mould count in pineapple puree treated at 400MPa/30 °C /10 min which was similar to the results obtained in the present study. The HPP treatment at 50 MPa can inhibit protein synthesis in microorganisms and reduce the number of ribosomes and at 100 MPa, leads to partial denaturation and at 200 MPa causes damage to cell membrane and internal cell structure.

Increasing the pressure to 300 MPa or more induce irreversible denaturation of enzymes and proteins causing rupture to the cell membrane and the excretion of internal substances resulting in microbial death (Abe, 2007). No coliform count was observed in

pressurized samples at both 300 MPa and 400 MPa for the dwell times of 10 to 25 min (Table 3). Hence, coliforms were found to be pressure sensitive compared to total bacteria and yeast & mould forms.

Microbial destruction effects of HP have been hypothesized to protein denaturation, cell membrane rupture and destabilization of cytoplasm contents due to increased membrane permeability which proves fatal for microbes (Meersman and Heremans, 2008). This may be the reason for microbial inactivation in HPP treated sugarcane juice.

Effect on physical properties

The effect of HPP on pH and TSS of sugarcane juice was summarized in table 4. The pH of the sugarcane juice was found to be in the range of 5.10 to 5.24, which determines the minimal effect of pressure on pH of the product. As High pressure processing does not affect the covalent and hydrogen bonding, the pH remained constant before and after the treatment and has no effect. The TSS of sugarcane juice varies from 19.42 to 21.42.

It was observed that with increase in pressure and dwell time, there is no significant effect on TSS of the sugarcane juice (Table 4). The change in colour *viz.*, CIE-*Lab* values of the HPP treated sugarcane juice have been presented in table 5. The ΔE^* values increased significantly with increase in pressure and dwell time for HPP treated sugarcane juice. The ΔE^* values under combined pressure and time for the treated samples varied from 0.25 to 1.67. Color changes were not visually noticed ($\Delta E < 2.5$) for pressurized juice at different dwell time.

It was observed that with increase in pressure from 300MPa to 400 MPa with constant dwell time, colour variation has increased

significantly (Fig1). Also, with increase in dwell time, ΔE values increased significantly within same pressure.

However, the colour change was observed to be below 2.5 and cannot be visually noticed as per the categorization suggested by (Cserhalmi *et al.*, 2006).

From the current study, high pressure processing at ambient temperature can be used as an effective technology to stabilize fresh sugarcane juice through reduction of microbial load and polyphenol oxidase activity thus preventing microbial spoilage with little changes in colour of sugarcane juice. However, further study has to be done to optimise the process parameters.

References

- Abe, F., 2007. Exploration of the effects of high hydrostatic pressure on microbial growth, physiology and survival: perspectives from piezophysiology. *Biosci. Biotechnol. Biochem.*, 71(10): 2347-2357.
- Anese, M., Nicoli, M. C., Gianfranco, D. and Lerici, C. R. 1994. Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *J. Food Biochem.*, 18(4): 285-293.
- Balny, C., and Masson, P. 1993. Effects of high pressure on proteins. *Food Rev. Internatl.*, 9(4): 611-628.
- Barros, A. M., Laboissier, L. H. E., Rosenthal, A., Camargo, L. M. A., Siqueira, R. S. and Chakraborty, S., Mishra, H. N. and Knorr, D. 2012. *Strawberry enzyme inactivation by HPP: Models & contours*. LAP LAMBERT Academic Publishing.
- Chauhan, O.P., Singh, D., Tyagi, S.M. and Balyan, D.K. 2002. Studies on preservation of sugarcane juice. *Int. J Food Properties*, 5: 217-29.
- Cserhalmi, Z., Sass-Kiss, Toth-Markus, M. and Lechner, N. 2006. Study of pulsed electric field treated citrus juices. *Innovative Food Science and Emerging Technologies*, 7(1-2): 49-54.
- Deliza, R., 2005. Pineapple purée processed using high pressure. In: *Enpromer*.
- Gross, M., and Jaenicke, R. 1994. Proteins under pressure. *European Journal of BioChemistry*, 212(2): 617-630.
- Hendrickx, M., Ludikhuyze, L., Van den Broeck, I. and Weemaes, C. 1998. Effects of high pressure on enzymes related to food quality. *Trends in Food Science & Technology*, 9(5): 197-203.
- Karthikeyan, J., and Samipillai, S. S. 2010. Sugarcane in therapeutics. *Journal of Herbal Medicine and Toxicology*, 4(1), 9-14.
- Meersman, F., and Heremans, K. 2008. High hydrostatic pressure effects in the biosphere from molecules to microbiology. In: Michiels c, Barlett D H and Aertsen A. (eds) *High Pressure Microbiology*. Washington, DC; ASM press, pp. 1-17.
- Qudsieh, H.Y.M., Yusof, S., Osman, A. and Rahman, R.A. 2002. Effect of maturity on chlorophyll, anin, colour and polyphenol oxidase (PPO) activity of sugarcane juice (*Saccharum officinarum* Var. yellow cane). *J. Agri Food Chem.* 50: 1615-1618.
- Yusof, S., Shian, L.S. and Osman, A. 2000. Changes in quality of sugarcane juice upon delayed extraction and storage. *Food Chem.* 68: 395-401.
- Zabetakis, I., Leclerc, D. and Kajda, P. 2000. The effect of high hydrostatic pressure on the strawberry anthocyanins. *J Agric Food Chem*, 48(7):2749-54.

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