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Optimization of process parameter combinations for pasteurization of sugarcane (*Saccharum officinarum*) juice using continuous flow microwave system

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ABSTRACT

Colour (L*, a*, b*) values of fresh sugarcane (*Saccharum officinarum* L.) juice added with citric acid, thermal pasteurized and microwave pasteurized juices were significantly higher from that of fresh sugarcane juice. There was no significant difference in TSS and pH values of any of the pasteurized sugarcane juice. PPO activity and total viable bacterial count decreased significantly after thermal and microwave pasteurization of juice whereas yeast was completely eliminated from all the microwave pasteurized and thermal pasteurized sugarcane juices. There was a significant decrease in total antioxidant values after thermal and microwave pasteurization.

Key words: Colour, Microwave heating, Pasteurization, Total antioxidants

Pasteurization of liquid foods using microwaves have been reported to result in excellent retention of nutritional and sensory value besides having other advantages like saving in energy, cost, time etc. (Cannumir *et al*. 2002). However, in order to maximize its benefits, it is essential to carefully optimize the process with respect to the characteristics of the food that is being treated, rate of heating required, thermal death time of the targeted microorganisms, time and temperature combinations to achieve desired reduction in the targeted microbial load, etc. Fresh sugarcane juice is a popular low cost sweet beverage relished for its sweet taste and flavour. However, juice from sugarcane is often extracted manually or mechanically by crusher and consumed immediately because of its low storability due to presence of polysaccharides, starches, waxes and proteins; and browning because of enzymatic degradation of chlorophyll (Zhao *et al.* 2011). Sugarcane (*Saccharum officinarum* L.) juice if preserved properly and its sugar composition modified suitably can also be promoted as a high fructose-oligosaccharides and low sucrose drink.

A systematic study was undertaken for pasteurization of sugarcane juice using continuous pasteurization system which uses microwave energy for heating the liquid food.

MATERIALS AND METHODS

Fresh sugarcane juice was extracted from sugarcane using a roller crusher before experimentation in the laboratory. The extracted juice was immediately mixed with citric acid (CA) @ 1 g/l to inhibit enzymatic browning and enhance flavour of juice. Sugarcane juice was subjected to time-temperature combination of 70˚C for 10 min. The treatment combination was adopted from previously reported literature (Chauhan *et al*. 2002 and Sangeeta *et al.* 2013) for sugarcane juice. Sugarcane juice was subsequently subjected to pasteurization using the developed system and was exposed to five different microwave power levels (210, 280, 350, 420 and 490 W) to attain the desired heating. Flow rate was adjusted using the peristaltic feed pump so that the pumped liquid could attain the desired final temperature of 70°C in a single pass at the end of the heating section. Heated sugarcane juice was then transferred to a constant temperature water bath for holding the heated juice for the desired time period of 10 min. The juice was then collected, cooled in an ice water bath and tested for bio-chemical, microbial and nutritional attributes. Conventionally heated sugarcane juice treated with the same time-temperature combination was used as control.

Hunter colour values (L*, a*, b*) were measured using a Hunter Lab System. The system was calibrated using reference tiles (black, white and green). After calibration, the liquid sample was evaluated using a cup, ring and disc accessory.

The total soluble solids (TSS) of sugarcane juice samples was measured at room temperature by using FISCHER digital hand refractometer having range 0 to 50 and results were expressed as degree brix (AOAC 1980).

The pH of sugarcane juice samples was measured at room temperature by using a digital pH meter after standardizing with standard buffers of pH 4.0, 7.2 and 9.2.

Polyphenol oxidase (PPO) enzyme activity of sugarcane juice was estimated by using method of Zhao

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et al. (2011). The raw and treated sugarcane juice samples were centrifuged at 11000 rounds per min at 2˚C for about 20 min. The pellet is discarded and enzyme extract was transferred to an amber colour glass wile and stored at -80˚C until further use within 24hr**.** The reaction mixture for the assay of PPO contains 0.7 ml of 0.05 M catechol, varying amount of enzyme extracts and 0.2 M phosphate buffer (pH 6.8) in a final volume of 3 ml. Absorbance was noted down at 410 nm at room temperature. Readings was recorded at the gap of 30 seconds and expressed as ΔA_{410} OD/min/ml.

The total antioxidant (TA) content in sugarcane juice samples was determined using CUPRAC (cupric ion reducing antioxidant capacity) method by Apak *et al*. (2004). To a test tube, 1 ml each of copper chloride, neocuprine and ammonium acetate buffer solution were added. After that 0.1 ml of antioxidant sample solution was added to it. Then 1 ml of distilled water was added to it to make the final volume up to 4.1 ml. After 30 min, the absorbance was recorded at 450 nm.

Sugarcane juice was pasteurized using the developed system and tested for the presence of viable bacterial count by dilution plating on nutrient agar media (Krishnakumar and Devadas 2006).

The experiment was conducted using a CRD design with 3 replications. Statistical analysis was done using analysis of variance (ANOVA) technique in the CRD (Panse and Sukhatme 1984). The data was analyzed using SAS software SPSS (1998).

RESULTS AND DISCUSSION

The responses with respect to L*, a*, b* values, total soluble solids (TSS) (˚B) and pH under study along with their standard deviation values are presented in Table 1.

Table 1 Effect of pasteurization (70˚C for 10 min) at different microwave power levels on L*, a*, b* values, total soluble solids (TSS) (˚B) and pH of sugarcane juice

Treatment	L*	a*	b*	TSS	pH
Fresh juice	23.14 ±0.45	1.64 ±0.09	15.71 ±0.35	12.4 ±0.1	5.29 ±0.04
Fresh juice + CA	36.03 ±0.75	3.86 ±0.57	22.69 ±0.57	12.43 ±0.05	4.12 ±0.01
Thermal pasteurized	37.01 ±0.42	4.71 ±0.42	24.24 ±0.06	12.36 ±0.11	4.10 ±0.01
210 Watt	38.86 ±0.59	5.92 ±0.23	24.59 ±0.30	12.4 ±0.1	4.12 ±0.01
280 Watt	39.64 ±0.29	6.12 ±0.32	24.92 ±0.43	12.36 ±0.15	4.12 ±0.04
350 Watt	39.80 ±0.13	6.36 ±0.38	25.08 ±0.79	12.4 ±0.26	4.11 ±0.026
420 Watt	39.94 ±0.44	6.58 ±0.38	25.18 ±0.37	12.43 ±0.15	4.10 ±0.015
490 Watt	40.78 ±0.34	6.88 ±0.43	25.27 ±0.14	12.43 ±0.15	4.11 ±0.02

Colour (L, a*, b* values)*

There was significant difference between L*, a*, b* values of fresh sugarcane juice, fresh juice added with citric acid (Fresh juice + CA @ 1 g/l), thermal pasteurized (control) and microwave pasteurized juices. L*, a*, b* values of fresh sugarcane juice added with citric acid, thermal pasteurized and microwave pasteurized juices were significantly higher from that of fresh sugarcane juice. Fresh sugarcane juice was having lower L value in comparison to all other treatments because fresh sugarcane juice immediately turned brown after extraction because of high polyphenol oxidase enzyme activity. Mao *et al*. (2007) also reported that PPO activity converts phenolic compounds into brown coloured polymers, which causes darkening when sugarcane is crushed. With the addition of citric acid (1g/l) to juice the pH of juice dropped to 4.1 which reduced the enzymatic activity and browning of juice and juice was lighter in colour. Kunitake *et al.* (2014) also reported that sugarcane juice added with passion fruit pulp act as an acidified beverage with pH below 4.6 which inhibited enzyme action especially the PPO enzyme which has an optimum working pH close to 7.2 (Gomes *et al*. 2001). Moreover, the citric acid in the passion fruit pulp complexes with the copper (Cu^{2+}) present in the PPO active site, therefore inhibiting enzymatic browning of the juice (Romano 2006). When this citric acid added sugarcane juice was further heated for pasteurization it inactivated the PPO enzyme and inhibited the browning of juice. Kunitake *et al*. (2014) also reported that processed sugarcane juice beverages presented higher L values than the unprocessed ones, indicating that they had a lighter colour. They also found that processing at (90 or 95°C) results in a significant increase in the L value when compared to the beverages processed at 85°C which they suggested was due to greater enzyme inactivation at the higher processing temperatures. Fresh juice added with citric acid and pasteurized sugarcane juice was having higher a and b values in comparison to fresh juice because of reduced enzyme activity and degradation of chlorophyll present in the juice due to heat respectively. Kunitake *et al*. (2014) found that despite inactivation of PPO during pasteurization alterations in color were still observed, suggesting that other mechanisms, like chlorophyll degradation also occur. L*, a*, b* values of microwave pasteurized juice increased with the increasing power level from 210 to 490 Watt. For all the microwave pasteurized juice samples, higher L*, a*, b* values were maintained in comparison to thermal pasteurization. Higher L value of microwave pasteurized juice in comparison to thermal pasteurization may be due to higher PPO enzyme inactivation with microwave due to some non thermal effects associated with microwave which increased with the increasing power level. Matsui *et al*. (2008) also reported a faster inactivation of polyphenol oxidase and peroxidase enzyme through microwave pasteurization of coconut water in comparison with conventional pasteurization. Higher a and b value of microwave pasteurized juice may be due to higher degradation of chlorophyll in microwave due to high power the available energy is too high to trigger deterioration of chlorophyll. Jittanit *et al*. (2011) reported that the color of UHT processed sugarcane juice significantly differed from the fresh counter part especially the redness (a*). The redness of UHT processed juice was higher than the fresh juice. The explanation was that the heat during sterilization expedited the maillard reaction between the reducing sugar and amino acid in the juice leading to the browning and degreening. Moreover, the degreening of UHT processed sugarcane juice could also be due to the decrease in chlorophyll content during the sterilization. Mao *et al*. (2007) also reported that degreening of sugarcane juice appeared with a rapid increase in a value. Lowest L*, a*, b* value of 23.14, 1.64 and 15.71 was for fresh sugarcane juice whereas highest L*, a*, b* value of 40.78, 6.88 and 25.27 was found for juice pasteurized at 490 Watt (Table 1).

Total soluble solids (TSS)

There was no significant difference in TSS values of fresh and any of the pasteurized sugarcane juice. TSS values in sugarcane juice varied between 12.36 to 12.43 °Brix (Table 1). The results were supported by the finding of Canumir *et al*. (2002) who reported that pasteurization with different power-time combinations does not have any negative effect on TSS content.

pH

There was no significant difference in pH values of any of the microwave pasteurized sugarcane juice; however, all the treated sugarcane juice were having significantly lower pH in comparison to fresh sugarcane juice. Picouet *et al*. (2009) also found that microwave treatments did not affect the pH of Granny Smith apple puree. Treated sugarcane juice was having significantly lower pH in comparison to fresh sugarcane juice because of addition of citric acid at the rate of 1 gm/litre to fresh sugarcane juice before pasteurization.pH values in sugarcane juice varied between 4.10 to 5.12 (Table 1). Lowest pH value of 4.10 was for thermal pasteurized whereas highest pH value of 5.29 was found for fresh sugarcane juice.

Polyphenol oxidase (PPO) enzyme activity

The initial PPO activity in fresh sugarcane juice was 1900.38 unit/ml (Fig 1). PPO activity in fresh sugarcane juice decreased significantly after the addition of citric acid. This effect may be due to the lowering of pH by the addition of citric acid. PPO activity in fresh sugarcane juice added with citric acid decreased significantly after thermal pasteurization and microwave pasteurization. Residual PPO activity decreased with the decreasing power level from 490 to 210 Watt. The obtained result of effectiveness of microwave pasteurization at lower power level was also supported by Math *et al*. (2014). Lowest residual PPO activity of 11.69% (222.33 unit/ml) was found at 210 Watt microwave power level pasteurized sugarcane juice whereas highest residual PPO activity of 59.18% (1124.76

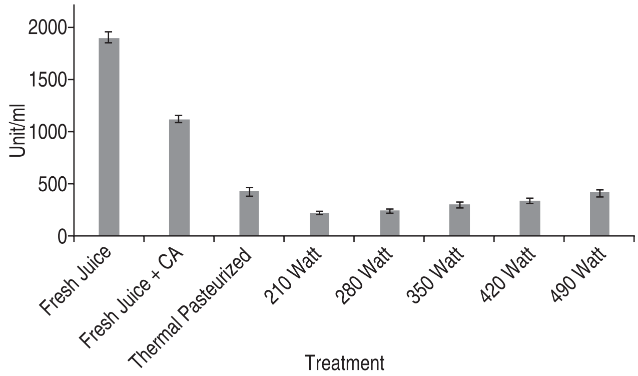

Fig 1 Effect of pasteurization (70°C for 10 minute) at different microwave power levels on polyphenol oxidase (PPO) enzyme activity of sugarcane juice

unit/ml) among the treated juice was of fresh juice added with citric acid. For all the microwave pasteurized juice samples, lower residual PPO activity was observed in comparison to thermal pasteurization. Matsui *et al*. (2008) also reported a faster inactivation of polyphenol oxidase and peroxidase enzyme through microwave pasteurization of coconut water in comparison with conventional pasteurization. Tajchakavit and Ramaswamy (1997) observed that the pectin methylesterase (PME) enzyme inactivation in orange juice was significantly faster in the microwave heating mode than in conventional thermal heating mode indicating some contributory nonthermal effects under the microwave heating conditions.

Total antioxidant content

The total antioxidant content in fresh sugarcane juice was about 10.78 μmol Trolox/ml (Fig 2). Total antioxidant content increased significantly after the addition of citric acid to fresh juice and with a value of 11.57 μmol Trolox/ml was found to be maximum among all the treatments. The preservative like citric acid has also been reported earlier to increase the antioxidant activity of processed food (Belitz *et al*. 2009). In comparison to fresh sugarcane juice added with citric acid, there was a significant decrease in total antioxidant values after thermal and microwave

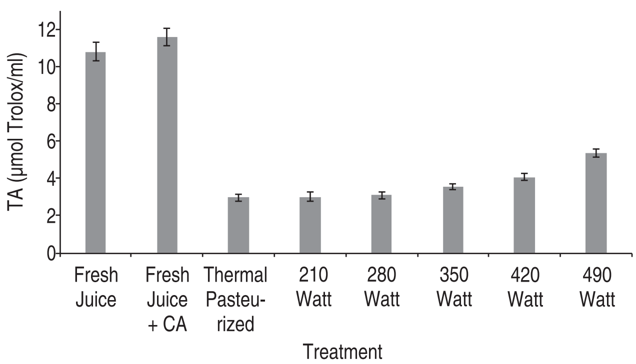

Fig 2 Effect of pasteurization (70°C for 10 min) at different microwave power levels on total antioxidant (μmol Trolox/ml) activity of sugarcane juice.

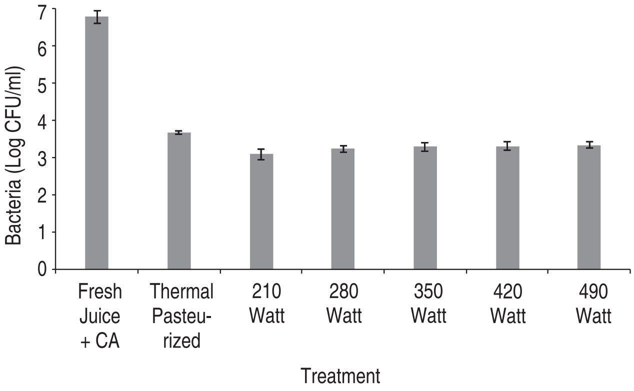

Fig 3 Effect of pasteurization (70°C for 10 minute) at different microwave power levels on microbial load (Log CFU/ml) of sugarcane juice.

pasteurizations. This decrease in total antioxidant values may be due to destruction of pigment compounds (chlorophylls and anthocyanins) present in sugarcane juice. Total antioxidant content retained increased with the increasing power level and all the microwave pasteurized samples were having higher antioxidant content in comparison to thermal pasteurized samples.Total antioxidant content retained increased with the increasing power level due to decreased heating time in microwave oven at higher power levels because of high power available per ml of juice. All the microwave pasteurized juice samples were having higher total antioxidant content in comparison to thermal pasteurized samples. This may be due to rapid and volumetric heating of juice in microwave oven in comparison to thermal pasteurization which help in retaining pigment compounds. With a value of 2.97 µmol Trolox/ml thermal pasteurized sugarcane juice was having least antioxidant activity among all the treated sample.

Microbial load

Total viable bacterial count in sugarcane juice added with citric acid decreased significantly after microwave and thermal pasteurization. The data depicted in Fig 3 revealed that total viable bacterial count decreased with the decreasing power level from 490 to 210 Watt. Higher microbial inactivation was observed at lower power levels due to increased heating time in microwave oven at lower power levels because of less power available per ml of juice. Lowest viable bacterial count of 3.09 Log CFU/ml was found at 210 Watt microwave power level pasteurized juice whereas highest viable bacterial count of 6.76 Log CFU/ml was of fresh juice added with citric acid. For all the microwave pasteurized juice samples, significantly lower viable bacterial count was observed in comparison to thermal pasteurization. This may be due to some non-thermal effects associated with microwave. This finding is consistent with the study of Nikdel *et al*. (1993), who reported that the microbial inactivation's in unpasteurized, conventionally pasteurized and microwave-pasteurized orange juice were 2×10^4, 550, and less than 10 CFU/ml, respectively. Koutchama *et al*. (2001) reported that microbial destruction occurs much faster under microwave heating than under thermal heating suggesting some enhanced effects associated with microwave heating.

Microwave pasteurization was found comparatively better method of pasteurization for sugarcane juice in comparison to thermal pasteurization for retaining the nutritional and functional qualities of sugarcane juice.

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