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Food Chemistry

Food Chemistry 104 (2007) 740-745

www.elsevier.com/locate/foodchem

Maintaining the quality of sugarcane juice with blanching and ascorbic acid

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Received 11 July 2005; received in revised form 21 September 2006; accepted 21 September 2006

Abstract

The physicochemical changes in fresh sugarcane juice stored at 10 °C were studied by determining juice yield, color, reducing sugar, titratable acidity, viscosity, pH, polyphenol oxidase (PPO), sucrose neutral invertase (SNI) and total microbial count. Results showed that blanching of stems before squeezing effectively prevented degreening and/or browning, and reduced activities of PPO and SNI in fresh sugarcane juice. Added ascorbic acid delayed the increase of reducing sugar, titratable acidity, viscosity and total microbial count, and also prevented degreening and/or browning with reduced PPO and SNI activities in fresh sugarcane juice during storage. Addition of 0.1% ascorbic acid seemed to be more effective than blanching of sugarcane stems, and was able to maintain the quality of fresh sugarcane juice for up to 5 days at 10 °C. Deterioration of fresh sugarcane juice was demonstrated as a rapid increase of titratable acidity and viscosity with a obvious browning.

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Keywords: Sugarcane; Juice; Blanching; Ascorbic acid

1. Introduction

Fresh sugarcane (*Saccharum officinarum*) juice is popular in many countries as a cheap and sweet beverage. It is becoming a fashion juice served at roadside stalls, cafeterias and restaurants throughout China during the harvest season. However, processing and marketing of sugarcane juice is limited by its rapid deterioration (Prasad & Nath, 2002; Yusof, Shian, & Osman, 2000). Development of effective treatments or procedures to keep the fresh quality of sugarcane juice would allow it to be more widely marketed, and would enhance its quality and safety as well.

Considerable efforts have been aimed at stabilizing the juice quality during processing and distribution. The most

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widely used method for delaying deterioration is blanching before juice extraction (Margherita & Giussani, 2003) and addition of antioxidant agents (Ozoglu & Bayindirli, 2002). Blanching treatment is usually performed by exposing vegetables or fruits to hot or boiling water for several seconds or minutes (Kidmose & Martens, 1999; Margherita & Giussani, 2003; Severini, Baiano, De Pilli, Romaniello, & Derossi, 2003). The most widespread antioxidant and acidify agent used in juice processing is ascorbic acid (Choi, Kim, & Lee, 2002; Pizzocarno, Torreggiani, & Gilardi, 1993). However, no study has been conducted to evaluate the possibility of blanching of sugarcane stems and addition of ascorbic acid to extend the shelf-life of sugarcane juice.

This study was conducted to evaluate the effectiveness of blanching and ascorbic acid in maintaining the quality of fresh squeezed and unpasteurized sugarcane juice in terms of color, reducing sugar, titratable acidity, pH, viscosity, polyphenol oxidase, sucrose neutral invertase activities and total microbial count.

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2. Materials and methods

2.1. Sugarcane juice

Mature stems of sugarcane (S. officinarum. cv. Badila) about 9 months old were cut close to the ground at a plantation in Hangzhou. Upon arrival at the laboratory, the stems were cleaned, hand-peeled and cut into three portions with equal length (about 50 cm). The middle portions were used for the experiment. Peeled stems were blanched by immersing in boiling water (40:1 ratio of water to stems, w/w) for 5 min and then drained and cooled prior to juice extraction. A self-made three-roller power crusher was used to extract the juice, which was filtered through an eight-layer muslin cloth. Ascorbic acid fortified juice was prepared by adding 0.1% (w/w) ascorbic acid to the juice. Juice was then filled into 260-ml glass bottles and stored at 10 °C immediately. Fresh juice extracted from unblanched stems and without addition of ascorbic acid was used as control. The experiment was performed in triplicate.

2.2. Juice yield and color

Sugarcane stems before squeezing and the extracted juice were weighted, respectively. Juice yield was calculated as the percentage rate of juice to stems. Color values of sugarcane juice were measured with a WSC-S colormeter (Precision Scientific Instrument Co., Ltd., Shanghai, China) by CIE-Lab color system. In previous study, we found greenness (a) and lightness (L) were the most sensible parameters to indicate visible color change in sugarcane juice.

2.3. Titratable acidity and pH

Titratable acidity was determined by quantifying the volume of 0.01 M NaOH required to raise the pH value to 8.3, and expressed as ml of 0.01 M NaOH per 10 ml of juice. Values of pH were measured with a digital pH meter (PHS-25, Precision scientific Instrument Co., Ltd., Shanghai, China). Buffers of pH 4.0 and 7.0 were used to standardize the equipment.

2.4. Viscosity and reducing sugar

The viscosity of juice was measured using a digital rheometer (L-90, Shanghai Tonji Science & Technology Industrial Co., Ltd, Shanghai, China) with spindle SC4-18 at 45 rpm (Ranganna, 1977). The viscosity of juice was evaluated according to the formula: η (Pa s) = $A \times$ 0.125. Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945), with glucose as the standard reducing sugar and 3,5-dinitrosalicylic acid as developer. The concentration of reducing sugar in juice was determined by using an equation that was obtained from standard glucose graph: $[C] = 5.58052 \times [A] - 0.11818$, $R^2 = 0.99814$.

2.5. Activities of polyphenol oxidase and sucrose neutral invertase

Polyphenol oxidase (PPO) reaction was started by adding 1 ml of 0.2 M catechol into the mixture containing 0.5 ml of sugarcane juice and 2 ml of 50 mM phosphate buffer (pH 6.5). Absorbance at every 1 min was recorded at 420 nm. One unit of PPO activity was defined as $0.001\Delta A_{420}$ /min (Ozoglu & Bayindirli, 2002). Neutral invertase (SNI) activity was determined according to Sehtiya and Densay (1991). The assay was started by addition 100 µl of 120 mM sucrose into the mixture containing 50 µl of sugarcane juice and 50 µl of 1 M sodium acetate (pH 7.5). The reaction was stopped at 60 min by boiling the mixture for 3 min. The concentration of glucose liberated was determined with the DNS method (Miller, 1959).

2.6. Microbiological analysis

Determination of total counts was based on Yusof et al. (2000). Serial dilutions $(10^{-1}, 10^{-2} \text{ and } 10^{-3})$ of sugarcane juice were prepared. One milliliter of each dilution was spread over the surface of plate count agar. The plates were then incubated for 48 h at 36 °C, and the count was defined as log cfu/ml.

2.7. Statistical analysis

Data were analysed by the analysis of variance and Duncan multiple range test using a statistical analysis system (SAS) programme.

3. Results and discussion

3.1. Juice yield

The yield of extracted juice was significantly decreased by the treatment of blanching. Juice yield in unblanched stems was $70.83 \pm 2.9\%$, but it decreased to $65.06 \pm 2.3\%$ when stems were previously blanched. This loss of 5% juice caused by blanching would be the result of leaching and leaking of juice while peeled sugarcane stems were dipped in boiling water.

3.2. Changes of color

Fresh sugarcane juice appeared olive-green and showed clear signs of degreening during processing and storage. Visually, juice extracted from unblanched stems was a little darker in color than that from blanched stems. Degreening appeared with a rapid increase of a value in sugarcane juice (Fig. 1a). However, blanching of sugarcane stems before squeezing and/or addition of 0.1% ascorbic acid significantly inhibited the occurrence of degreening in juice during storage. Combination of blanching of stems and addition of ascorbic acid showed an enhancive effect in preventing color change by indicating the lowest a value espe-



Fig. 1. Color *a* (a) and *L* (b) values in sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).

cially during the late period of storage. Browning was observed in the control with a rapid decrease of L value within the first 2 days. Afterward, juice color became lighter with increasing L values (Fig. 1b). This result indicated that blanching and ascorbic acid inhibited browning. The increase of L values during the late period of storage would be related with sedimentation of brown compounds.

3.3. Titratable acidity and pH

Titratable acidity in extracted juice increased rapidly during the late period of storage (Fig. 2). The increase occurred after 4 days of storage for samples without addition of ascorbic acid. Addition of 0.1% ascorbic acid reduced the increase of titratable acidity in juice from unblanched stems, and prevented this increase in juice from blanched stems.

Addition of ascorbic acid decreased the pH value by an average of 1.05 (Fig. 3). There were little changes of pH values in fresh sugarcane juice until day five. On the last day of storage pH values decreased by 8.4% in juice added with ascorbic acid, but by 27.7% when ascorbic acid was



Fig. 2. Titratable acidity of fresh sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).



Fig. 3. pH values of fresh sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).

not added. The increase of acidity was concomitant with the decrease of pH value, which could be due to the production of acetic acid and lactic acid in microbial growth (Bhupinder, Sharma, & Harinder, 1991). This study indicated that ascorbic acid had the significant effect in restraining the microbial growth in sugarcane juice.

3.4. Reducing sugar content

There was a slight increase of reducing sugar content in unpasteurized sugarcane juice during storage at 10 °C (Fig. 4). The greatest rate of increase was observed in the control juice with a 59.4% increase by the end of storage. Reduced increase of reducing sugar content in juice from blanched stems might be due to the fact that cell respiration and SNI activity are inhibited or slowed down by high temperature during blanching. Besides, reduced increase of reducing sugar that occurred in juice added with ascorbic acid was perhaps the result of reduced activity of SNI by



Fig. 4. Reducing sugar content of fresh sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).

decreasing of pH value below the optimum range of SNI activity.

3.5. Juice viscosity

Changes in juice viscosity were similar as acidity and reducing sugar content during storage at 10 °C. A rapid increase in viscosity was only appeared in juice without addition of ascorbic acid after 4 days of storage. Viscosity in juice added with ascorbic acid remained quite stable during the entire storage (Fig. 5). The increase in viscosity of juice might be due to the formation of dextran, i.e. a gummy substance produced by bacteria (Lotha, Khurdiya, & Maheshwawi, 1994; Yusof et al., 2000). Ascorbic acid may have retarded the growth of organisms, thus causing a low level and delay in the increase of viscosity.

3.6. Polyphenol oxidase activity

As shown in Fig. 6, both blanching of stems and addition of ascorbic acid could significantly reduce PPO activ-



Fig. 5. Viscosity of fresh sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).



Fig. 6. PPO activity of fresh sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).

ity in fresh sugarcane juice, and the effect of blanching was much more significant than addition of ascorbic acid. PPO activity in fresh sugarcane juice from unblanched stems was about sevenfold higher than that from blanched stems. On the other hand, addition of 0.1% ascorbic acid caused 12.7% decrease of PPO activity. Blanching treatment together with addition of ascorbic acid caused the lowest PPO activity, which was hardly detected throughout the storage. The highest PPO activity was observed in the control juice throughout the storage. There were little changes of PPO activity in all treated samples during storage.

High temperature during blanching inactivated PPO, which confirmed the observation of Vámos-Vigyázó (1981) who reported that PPO enzymes are destroyed at 80 °C although they are relatively heat labile. Ascorbic acid is a reducing compound and can be easily oxidized, which might reduce browning through preventing and/or reversing the oxidation of o-diphenols to o-quinones. These inhibitory effects of blanching and ascorbic acid on PPO activity were significantly related with the prevention of degreening and/or browning (Fig. 1). Browning is mostly the result of the activity of PPO enzyme acting on phenolic compounds to produce dark colored polymers when sugarcane is crushed to release the juice (Vickers et al., 2005). However, a slight degreening still happened in juice with no detectable PPO (from blanched stems and added with ascorbic acid), especially during the late period (Figs. 1a and 6). This result suggested that degreening of sugarcane juice would be related much more with chlorophyll degradation than with browning. Chlorophyll degradation is involved with the activity of several enzymes including chlorophyllase and pheophorbide *a* oxygenase (Takamiya, Tsuchiya, & Ohta, 2000).

3.7. Sucrose neutral invertase (SNI)

There was an obvious decrease of SNI activity in sugarcane juice during storage. Both blanching of sugarcane stems and addition of ascorbic acid reduced the SNI activities (Fig. 7). Sugarcane juice extracted from unblanched stems and without addition of ascorbic acid had the highest activity of SNI. Blanching with high temperature would destroy SNI protein. Optimum pH range for SNI is 7.0-8.0 (Liu & Zhu, 2002). Addition of ascorbic acid lowered the pH value far below the optimum range (Fig. 3) and thus reduced SNI activity. Sucrose breakdown could be mediated through both sucrose synthase and SNI. However, SNI is observed to play a major role in cleaving sucrose to glucose and fructose (Bosch, Grof, & Botha, 2004). This study indicated that a considerable activity of SNI still retained in extracted sugarcane juice and would play an important role in sucrose degradation, causing the increase of reducing sugar (Fig. 4). In addition, this study also demonstrated that inhibited SNI activities obviously contributed to the reduced increase of reducing sugar that occurred in juices from blanched stems and/or added with ascorbic acid (Figs. 4 and 7).



Fig. 7. Activities of sucrose neutral invertase in fresh sugarcane juice stored for 0 day and 4 days at 10 °C, respectively. Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (unblanching + AA) or without the addition of ascorbic acid (unblanching). Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (blanching + AA) or without the addition of ascorbic acid (blanching).



Fig. 8. Total microbial count in fresh sugarcane juice during storage at 10 °C. Juice extracted from blanched sugarcane stems and added with 0.1% ascorbic acid (\triangle) or without the addition of ascorbic acid (\triangle). Juice extracted from unblanched sugarcane stems and added with 0.1% ascorbic acid (\bigcirc) or without the addition of ascorbic acid (\bigcirc).

3.8. Total microbial count

There were significant influences of ascorbic acid and blanching on total microbial counts in stored sugarcane juice during storage (Fig. 8). There was an increase in viable count in juice during storage. Addition of ascorbic acid reduced total microbial count by an average of log 2.28 cfu/ml, but blanching before squeezing showed similar effect when juice was stored after 5 days and no ascorbic acid was added. Addition of ascorbic acid and blanching may have retarded the growth of organisms. This result could explain the increases of titratable acidity (Fig. 2) and viscosity (Fig. 5), and the decrease of pH value (Fig. 3) in juices without addition of ascorbic acid, especially when stems were not blanched.

4. Conclusion

Blanching of stems before squeezing reduced the juice yield by about 5%. Both blanching of stems and addition of ascorbic acid influenced all parameters determined. Both treatments had significant effects on preventing color change, delaying the increase of reducing sugar, titratable acidity, viscosity and total microbial count, reducing PPO and SNI activities. Freshly extracted, unpasteurised sugarcane juice could be kept at 10 °C for 5 days. Beyond that, the quality deteriorated as indicating browning, increased titratable acidity and viscosity. The combination of blanching of sugarcane stems and addition of ascorbic acid would produce the best quality of sugarcane juice.

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