Preservation of Sugarcane Juice by Canning 1. Effect of Thermal and Chemical Pre-treatments on the Enzymatic Browning of Sugarcane Juice

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Abstract: The enzymatic browning changes in fresh sugarcane juice stored at room temperature 25 ^{o}C and at refrigerator 4 ^{o}C were studied by determining juice colour as a capacity of browning and polyphenol oxidase (PPO) enzyme activity. Results showed that thermal and chemical pretreatments of stems before squeezing effectively prevented degreening and/or browning, and reduced activities of PPO of fresh sugarcane juice. Added citric acid and SO_2 prevented degreening and/or browning with reduced PPO enzyme activity in fresh sugarcane juice during storage at room temperature or at refrigerator. Addition of SO_2 seemed to be more effective than other chemical and thermal pretreatments of sugarcane stems, and was able to maintain the quality of fresh sugarcane juice for up to 15 weeks at 4 ^{o}C. Deterioration of fresh sugarcane juice was demonstrated as a rapid increase of polyphenoloxidase enzyme activity and with an obvious browning. [Journal of American Science 2010; 6(9):883-888]. (ISSN: 1545-1003).

Keywords: Sugarcane; Juice; steam; autoclave; citric acid, sodium metabisulphite, colour, PPO, browning

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 and Nath, 2002 and Yusof et al., 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 widely used method for delaying deterioration is blanching before juice extraction (Margherita and Giussani, 2003 and Lin et al., 2007) and addition of antioxidant agents (Ozoglu and Bayindirli, 2002). Blanching treatment is usually performed by exposing vegetables or fruits to hot or boiling water for several seconds or minutes (Kidmose and Martens, 1999 and Severini et al., 2003). The most widespread antioxidant and acidify agent used in juice processing is ascorbic acid (Lin et al., 2007; Choi et al., 2002 and Pizzocarno, et al., 1993). Enzymes activity (especially polyphenoloxidase enzyme activity) is a major problem for the food industry, since it causes deleterious changes in appearance and organoleptic properties of fruits and vegetables and, therefore, also a decrease in market value (V´ AMOS 1981, Labuza et al 1992 and Monsalve-Gonzalez et al 1995). Consequently, control of enzymatic browning has gained a lot of interest in food industries (Weemaes et al, 1998).

The use of chemical that lower the product pH, or acidulant, finds widespread application in the control of enzymes activity. In addition, there are variations in the effect of different acids on enzymes activity; as an example, malic acid and citric acid has been reported to be more efficient in the inhibitoion of enzyme activity (Nicolas et al, 1994). Ascrobic acid is probably the most widely used an inhibitors of enzymes activity, and in addition to its reducing properties, it also slightly lowers pH (Whitaker 1994 and Lin et al., 2007). Dipping is an important and necessary pretreatment in process fruits; it used to prevent enzymatic browning caused by poly phenol oxidase. Several chemicals including ascorbic acid (A.A), citric acid (C.A) and sodium meta-bisulphite have shown the capability of prevent enzymatic browning caused by poly phenol oxidase.. However, no study has been conducted to evaluate the possibility of thermal pretreatments of sugarcane stems and addition of citric acid and SO_2 to extend the shelf-life of sugarcane juice.

The main problem with sugarcane juice production is the assurance of colour, polyphenoloxidase enzyme activity and maintains colour palatability This study was conducted to evaluate the effectiveness of thermal and chemical pretreatments in maintaining the quality of fresh squeezed and un-pasteurized sugarcane juice in terms

of colour as a brown capacity and polyphenoloxidase activity during storage at room temperature (25°C) for 24 hours and at refrigerator (4°C) for 15 weeks.

2. Materials and Methods:

Sugarcane juice Processing

Mature stems of sugarcane (*Saccharum officinarum. cv. Badila*) were cut close to the ground at a plantation in Dokki, Cairo, Egypt. 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 thermal treated by immersing in steam for 10 minutes, autoclave at 126 °C and 121°C for 10 minutes (40:1 ratio of water to stems, w/w) 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 two-layer cheese cloth. Citric acid and sodium meta bisulfate (SO_2) fortified juice was prepared by adding 0.5, 1 and 2% (w/w) citric acid and 0.05, 0.1 and 0.3% (w/w) SO_2 to the juice. Juice was then .filled into 250-ml glass bottles and stored at room temperature 25°C or at refrigerator 4°C immediately. At all chemical treated samples, 0.1% potassium sorbate was addition to prevent any microbial growth during storage. Fresh juice extracted from untreated stems with addition of 0.1% potassium sorbate and without other addition of chemical compounds was used as control. The experiment was performed in triplicate.

Evaluation of brown capacity

Portions of thermal (steam, 121/10min and 126/10min autoclave) and chemical treated with different concentrations (citric acid and SO2) sugar cane juices and untreated control (25ml in 50ml beakers containing magnetic stirrer bars, covered to prevent evaporation), were stirred at 400 rpm for as long as 1440 min (24hr) at room temperature on a stirrer to accelerate any available enzymatic browning if it were to occur. Sugar cane Juices were held at room temperature with stirring during which time tristimulus reflectance L*-, a*- and b*-values for controls and treated juices were periodically measured during 24hr, using a spectrocolourimeter (Hunter, LabScan XE, Reston VA, USA) calibrated with a white standard Tile of Hunter Lab Colour Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16). The percentage difference between the untreated sample (control) and treatment delta a*-values after a specified storage time (t) was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Delta a* control - Delta a* treatment}}{\text{Delta a* control}}$$

Where: Delta a* is the difference between the*-value at time (t) and the initial value. Since the initial a*-values used as the basis for calculating delta a*-values were read at 1 min (Sapers and Douglas, 1987). Also, the tristimulus reflectance L*-, a*- and b*-values for controls and treated juices were periodically measured every week during storage at 4°C for 15 weeks.

Polyphenol oxidase enzyme activity determination:

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 0.001DA420/min (Ozoglu & Bayindirli, 2002).

Statistical analysis:

However, the experiment was performed in triplicate. The results were analyzed statistically using standard deviations, analysis of variance and Least Significant Difference (LSD) as described by Richard and Gouri (1987).

3. Results and Discussion:

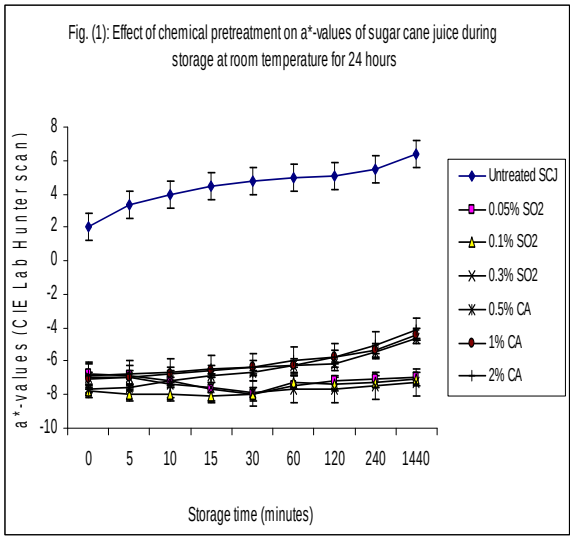
Effect of chemical and thermal pretreatments on enzymatic browning in sugar cane juice storage at room temperature and at refrigeration:

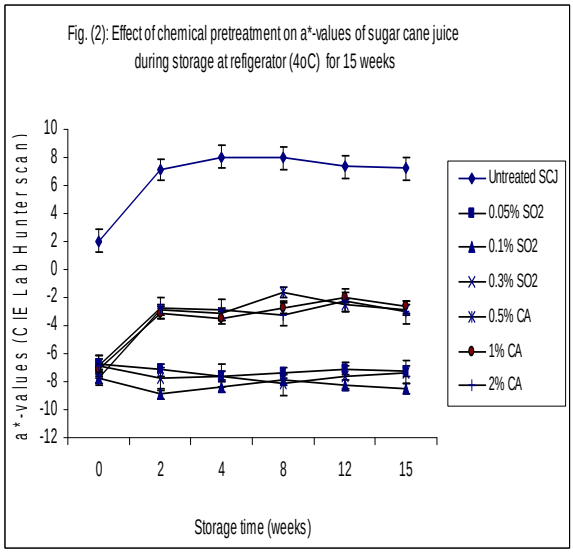
Raw sugar cane juice might represent a more useful system than the cut surface of plugs for the comparison of multilevel treatments to inhibit browning, since it would be homogeneous and more easily manipulated. However, preliminary experiments with a chemical (sodium meta bi sulphite (SO_2) and citric acid (CA) and thermal (steam and autoclave) pretreatments on sugar cane juice indicated that browning in the freshly prepared juice occurred too rapidly to permit sample treatment and evaluation. Reflectance measurements and a*-values were increased in the browning juices (Fig.1). Browning of the sugar cane juices was measured by a* (green-red). An increase in a*-value is indicative of browning (Monsalve, et al, 1993). No heat pretreatment was given to the fresh sugar cane juices; thus enzymatic activity of polyphenoloxidase was assumed. The results that show the effect of treating sugar cane juices with the different chemical as anti-browning agents and thermal pretreatment and storage at room temperature (25°C) for 24 hours and for 4 weeks at 4°C on inhibiting the browning reactions are graphically represented in Figs (1 & 2).

This figures illustrates the changes in the colour of sugar cane juices in terms of a*-values over

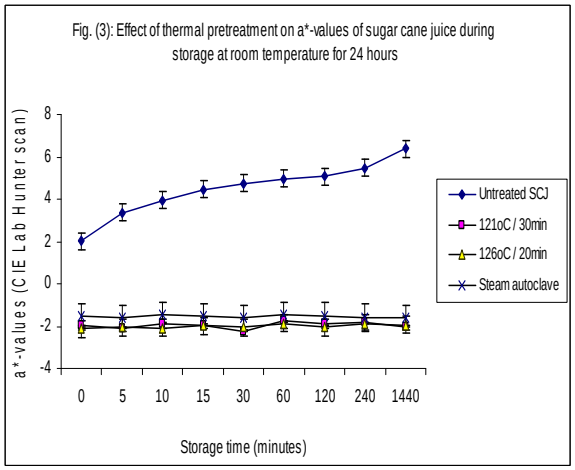
24 hours after thermal (steam and autoclave) adding of SO_2 at concentration 0.05, 0.1 and 0.3% and citric acid at concentration 0.5, 1 and 2% for each one. Also, the colour a*-values were recorded for the untreated sugar cane juice over 24 hours directly after preparing.

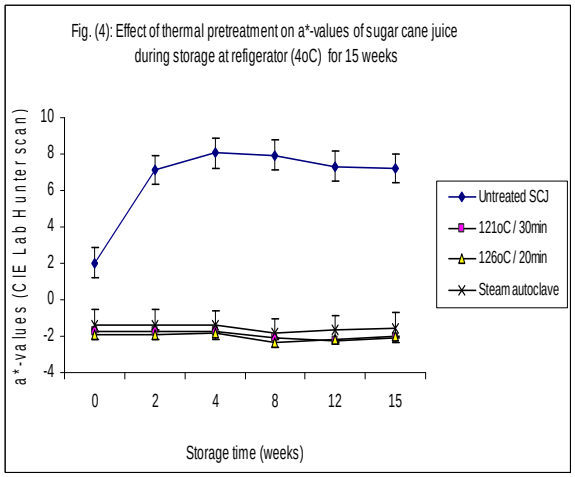
It can be observed that the sugar cane juices pretreated with chemical (sodium meta bi sulphite (SO_2) and citric acid (CA) and thermal (steam and autoclave) have no browning or the lowest a*-value (< -8.12) after 24 hours stored at room temperature (25^{o}C). While browning or a*-value of untreated sugar cane juices was too high (>6.38) after 24 hours stored at room temperature (25^{o}C). On other hand, both SO_2 and CA are considered to be anti-browning agents in controlling enzymatic browning reactions, as seen in fig. (1).





Furthermore, for long-term storage at 4^{o}C, the results showed that the sugar cane juices treated with chemical (sodium meta bi sulphite (SO_2) and citric acid (CA) and thermal (steam and autoclave) had no browning or the lowest a*-value (<-8.87 to -2.29) after 4 weeks stored at refrigerator (4^{o}C). While the a*-value of untreated sugar cane juices was too high (7.22) after 4 weeks stored at refrigerator (4^{o}C), as seen in Fig 2. The results showed that application of a browning inhibitor solution containing chemical (sodium meta bi sulphite (SO_2) and citric acid (CA) and thermal (steam and autoclave) could control the enzymatic browning of sugar cane juices. The use of these pretreatments, especially SO_2, CA and refrigeration at 4^{o}C for 4 weeks constitutes an effective method of quality improvement and shelf life extension. However, refrigerating and chemical or thermal pretreatments caused inhibition of enzymatic browning up to 4 weeks at 4 ^{o}C and maintained quality of sugar cane juice, as seen in figs (3 and 4).





The most effective chemical and thermal pretreatments were SO_2 and autoclave of sugar cane juices stored for 24 hours at 25°C and stored for 4 weeks at 4 °C (Figs 1 - 4). These results indicated that the chemical and thermal pretreatments inhibited browning of refrigerated sugar cane juices compared with that of the untreated samples.

From the above mentioned results it could be concluded that the pretreated sugar cane juice with sulphites (SO_2) had the best colour values (a* values) and lower non-enzymatic browning compared to the other pre-treatments especially in citric acid and thermal treated and untreated of sugar cane juice, as seen in Figs (1 to 4). The most effective chemical treatments for the inhibition of enzymes, good colour characteristics and lower non-enzymatic browning in plum fruits juice during storage was SO_2 (0.1%) and citric acid (0.5%).

Effect of thermal and chemical pretreatments on changes of colour characteristics in sugar cane juice

Fresh sugarcane juice appeared olive-green and showed clear signs of degreening during processing and storage at room temperature 25 °C and at refrigerator 4 °C. Visually, juice extracted from untreated stems was a darker in colour than that from treated stems. Degreening appeared with a rapid increase of a*-value in sugarcane juice (Figs. 1-4). However, thermal pretreated of sugarcane stems before squeezing and/or addition of citric acid and SO_2 significantly inhibited the occurrence of degreening in juice during storage. Both of thermal of stems and addition of citric acid and SO_2 showed an enhancive effect in preventing colour change by indicating the lowest a*- value especially during the late period of storage at room temperature 25 °C and at refrigerator 4 ° C. Browning was observed in the control with a rapid increase of a*- value within the first 5 days. Afterward, juice colour became lighter with decreasing a*- values during storage at room temperature (Fig. 1-4). This result indicated that thermal and chemical pretreatments inhibited browning. The decrease of a*- values during the late period of storage would be related with sedimentation of brown compounds.

Effect of thermal and chemical pretreatments on Polyphenol oxidase enzyme activity in sugar cane juice

As shown in Table (1), both thermal (steam and autoclave) stems and addition of SO_2 and CA could significantly reduce PPO activity in fresh sugarcane juice, and the effect of chemical pretreatments with high concentration pretreatments was much more significant than thermal pretreatments. PPO activity in fresh sugarcane juice from untreated stems was about hundredfold higher than that from chemical and thermal treated stems. On the other hand, addition of 0.0.05% SO_2 and 0.5% CA caused 4.5 and 10.6% decrease of PPO activity. Thermal and chemical pretreatment caused the lowest PPO activity, which was hardly detected throughout the storage. The highest PPO activity 0.571 (Unit / ml juice) was observed in the control juice, hence there were high changes of PPO activity in all treated samples.

Table (1): Effect of thermal and chemical pretreatments on polyphenoloxidase enzyme activity in sugarcane juice.

SCJ Samples	Unit / ml juice	% activity	% inhibition
Control SCJ	0.571 (±0.06)*	100	0
0.05% SO_2	0.02 (±0.14)*	3.502627	96.49737
0.1% SO_2	0	0	100
0.3% SO_2	0	0	100
0.5% CA	0.055 (±0.09*)	9.632224	90.36778
1% CA	0	0	100
2% CA	0	0	100
AU 121 °C	0	0	0
AU 126 °C	0	0	0
Steam	0	0	0

* standard deviations

High temperature during thermal pretreatments inactivated PPO, which confirmed the observation of Va´mos-Vigya´zo´ (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. But, these inhibitory effects of thermal (steam and autoclave) and chemical (SO_2 and CA) pretreatments 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 coloured 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 thermal treated stems and added with citric acid and SO_2), especially during the late period (Figs. 1 to 4). 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 et al., 2000).

4. Conclusion:

Thermal and chemical pretreatments of stems before squeezing reduced the juice browning by 100%. Both thermal of stems and addition of citric acid and SO_2 influenced all parameters determined. Both pretreatments had significant effects on preventing colour changes and reducing PPO activities. Freshly extracted, unpasteurised sugarcane juice could be kept at 4 ^oC for 15 weeks. Beyond that, the quality deteriorated as indicating browning and PPO inhibition. The thermal of sugarcane stems and addition of citric acid and SO_2 would produce the best colour characteristics quality of sugarcane juice. Finally, it could be concluded that thermal and chemical pretreatments of sugar cane stems such as soaking in citric acid and SO_2 for 30 min before storage at 4 ^oC for 15 weeks, improved its colour characteristics and enhanced the extracted juice colour quality regarding inactivation of PPO enzymes activities resulting higher shelf-life and sensorial properties, especially in sugar cane stems treated with SO_2 of sugar cane juice but in sugar cane treated with citric acid regarding colour properties of sugar cane juice. In consequence, the sugar cane juice could be considered a strong product that includes a "juicy aroma" in accordance with consumer expectation for juice

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