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Original Article

Physicochemical changes of Malaysian red Ragnar sugarcane juice treated with chemical preservatives during storage

Nusrah Mansor, Sarina Abd-Halim, Lee Pick Wui, and Muhamad Hafiz Abd Rahim*

Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, 43400 Malaysia

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Abstract

This study aimed to determine the best chemical preservative for preserving the quality profiles of the Red *Ragnar* sugarcane juice. The preservation was performed by using various types and concentrations of the chemical preservatives, namely sorbic acid (SA), sodium benzoate (SB), potassium sorbate (PS), ascorbic acid (AA), and citric acid (CA) under room temperature for 7 days. The treatments led to a pH reduction like the control (3.31 on day 7), except for the 0.8% CA treatment that had a significantly lower pH (2.67). 0.8% CA also demonstrated the largest °Brix reduction of 40.79%, followed by 0.8% AA at 31.57%. The colour profile was improved in all samples, indicated by lighter (ΔL), greener (Δa), and bluer (Δb) colour than the control. 0.8% AA was found to be the best enzymatic anti-browning agent (97.18% PPO activity reduction) followed by 0.1% PS (77.88%). PS was the most effective in reducing microbial presence, as only an average of 10^4 microorganisms was detected at all concentrations. All the treatments were non-toxic, as their median lethal concentration (LC_{50}) did not exceed 1000 µg/ml. These observations indicate that PS might be the best preservative for red sugarcane juice due to its excellent ability to inhibit microbes and browning.

Keywords: sugarcane, preservative, shelf-life, physicochemical, browning

1. Introduction

Sugarcane is one of the tallest grass families of *Saccharum*, and it can grow up to 14 feet tall in tropical areas. There are many varieties of sugarcane planted in Malaysia; most of them are cultivated for sugar production while some part is for juice and bioconversion purposes (Hajar-Azhari *et al.*, 2020, 2021).

. These varieties are usually recognized based on their rind color. To produce juice, sugarcane is generally pressed and crushed, which is common in countries where sugarcane is grown commercially (Qudsieh *et al.*, 2002).

The shelf life of sugarcane juice is often limited due to its unfavorable composition with high microbial contamination, low acidity, high water activity, and high sugar content (Aneja, Dhiman, Aggarwal, & Aneja, 2014a; Frazier & Westhoff, 1978). Additionally, the darkening of sugarcane juice after extraction will occur due to the formation of brown pigments by enzymatic reactions (peroxidase, POD, and polyphenol oxidase, PPO) and non-enzymatic reactions (Maillard reaction) where the oxidation of phenolic compounds leads to the formation of melanin (Qudsieh *et al.*, 2002). Both of the enzymes can be denatured by heat, reducing their activity, and thus improving the stability of the product. However, as heat always leads to the destruction of the heat-sensitive bioactive compounds, this method is usually undesirable (Jasmi *et al.*, 2020).

The most common commercial variant of red sugarcane in Malaysia is *Ragnar*, which possesses sweet and highly aromatic juice (Azhari, Shahruddin, & Abd Rahim, 2018; Mansor, Ramli, Azhari, & Abd Rahim, 2020). Due to its lesser popularity compared to yellow sugarcane, a proper methodology is yet to be established to extend and optimize its shelf life. Currently, there are a variety of permitted chemical preservatives that could be used to inhibit the growth of microorganisms (Khan *et al.*, 2021). As most of the sugarcane juice is sold by small-time roadside hawkers, it is paramount to preserve the juice quickly in an affordable and

*Corresponding author
Email address: muhdhafiz@upm.edu.my

accessible manner. Therefore, the use of chemical/weak-acid preservatives in allowable quantities is the best solution to the problem. Typically, these chemical preservatives reduce the pH rapidly and retard the growth of microorganisms by changing the physiological properties of the cell membrane, transport, and enzyme systems (Stopforth, Sofos, & Busta, 2005). The use of ascorbic and citric acids has been shown to increase shelf life, preserve the color, reduce activities of PPO and sugarcane neutral invertase (NI) as well as reduce the viscosity and total microbial count in sugarcane juice (Mao *et al.*, 2007; Mishra *et al.*, 2011). Likewise, the use of benzoates and sorbates was able to reduce total soluble solids, pH, and total sugar, which lead to significantly longer shelf life for fruit juices (Aneja *et al.*, 2014a; Pylypiw & Grether, 2000).

In this investigation, the freshly extracted red sugarcane juice of the *Ragnar* variant was subjected to varied concentrations and types of chemical preservatives (sorbic acid (SA), sodium benzoate (SB), potassium sorbate (PS), ascorbic acid (AA), and citric acid (CA)) for storage under room temperature. The impact of these chemicals on pH, total soluble solids (°Brix), colorimetric coordinates (L*, a*, and b*), polyphenol oxidase (PPO) activity, non-enzymatic browning index (NEBI), total plate counts, yeast and molds count, and median lethal concentration (LC_{50}) will be discussed. These outcomes are essential determinants of freshness, safety, and shelf-life, and the results can be used to aid the hawkers processing sugarcane juice at room temperature.

2. Materials and Methods

2.1 Preparation of samples

Red *Ragnar* sugarcane juice was procured from a small farm located in Pendang, Kedah, Malaysia, after 6 months of cultivation. All the chemicals and reagents used in this study were of analytical grade.

The concentrations of SB, PS, and SA used were 0.1% and 0.2%, while the concentrations of CA and AA used were 0.4% and 0.8%. A control sample without added preservatives was prepared. Treated samples were mixed well and kept in pre-sterilized universal bottles. All of the treated samples were stored at room temperature (on average 26°C) for 7 days before analyses of their physicochemical, microbiological, and toxicology properties.

2.2 Physicochemical and enzymatic determinations

pH was determined by using a pH meter (Jenway 3505). The total soluble solids (°Brix) was determined with a handheld refractometer (Atago). Color was determined by using a colorimeter (HunterLab) providing the color coordinates lightness (L*), redness/greenness (a*, ± red-green), and yellowness/blueness (b*, ± yellow-blue). "Δ" indicates the difference between an actual treatment sample and the control. An increase in ΔL indicates an increase in darkness, an increase in Δa indicates the color is turning green from red, and an increase in Δb indicates the colour is turning blue from yellow (Whetzel, 2016).

The polyphenol oxidase (PPO) activity was determined by adding 0.5 mL of the treated sample with 2 mL of 0.5 M phosphate buffer (pH 6.5). Then, the sample was centrifuged at 5,000 rpm for 10 mins. The supernatant was collected and mixed with 1 mL of 0.2 M catechol. The absorbance was then read at 420 nm at 1 min interval for 20 mins by using a spectrophotometer (Genesys 20). The results are expressed in $U.mL^{-1}$, which is a common unit for enzyme activity.

The non-enzymatic browning index (NEBI) was determined based on (Azhari *et al.*, 2018). 5 mL of 95% ethyl alcohol was added to 5 mL of sample and centrifuged at 5,000 rpm for 10 mins. The supernatant of the centrifuged sample was measured for absorbance at 420 nm using a spectrophotometer. The absorbance obtained was recorded as the NEBI.

2.3 Microbiological and toxicological activity determination

Plate Count Agar (PCA) was prepared according to the manufacturer's recommendation. The logarithms of colony-forming unit counts per gram (log_{10} CFU/g) for the samples were calculated by observing and enumerating the total colonies formed after incubation at 35°C for 48 h on PCA agar. Similar procedures were performed for yeast and mold determination, using Potato Dextrose Agar (PDA) media.

A brine shrimp lethality test was performed to measure the toxicity of the preservatives. Concentrations of 10^{-1}, $10^{-2,}$ and 10^{-3} were prepared by serial dilution from the treated sample. Seawater solution (negative control) was prepared by adding 38 g of NaCl to 1 L of distilled water. The number of dead brine shrimps *naupii* was calculated and recorded after 24 h. The percentage of mortality for each dose was determined by comparing the mean surviving *naupii*. LC_{50} values were obtained from the best-fit line to concentration versus percentage mortality.

2.4 Statistical analysis

MINITAB application software (Version 16.0) was used to perform the analysis of variance (One-Way ANOVA). During this analysis, Tukey's test was used to determine the significance of the difference ($P < 0.05$) between treatments.

3. Results and Discussion

3.1 Physicochemical and enzymatic changes after treatments

The pH is one of the important sensory properties and influences the rate of deterioration of sugarcane juice. All treatments on day 7 displayed significantly lower pH compared to the initial pH of control on day 0, indicating that the addition of preservatives managed to lower the pH as intended (Figure 1(a)). This outcome would be beneficial in inhibiting deterioration, but is undesirable in terms of preserving the natural sensory character of the juice. Looking at the individual preservatives, 0.2% PS decreased pH the most (2.42 difference) and 0.4% CA decreased it the least (0.04 difference) when compared to the initial pH on day 0. The increase in acidity (decrease in pH) might be due to the formation of acidic compounds from reducing sugar, or to the growth of acid-producing bacteria (Aneja *et al.*, 2014).

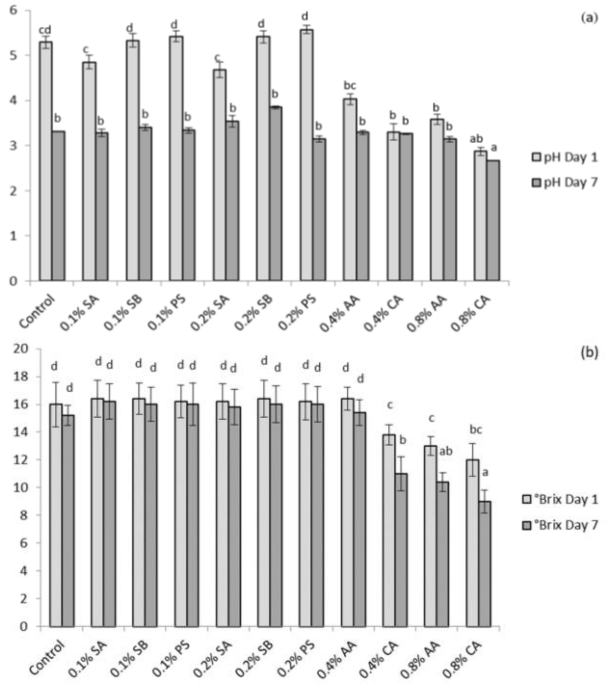

Figure 1. (a) The change in pH, and (b) the change in °Brix caused by the addition of preservatives. Different superscripts indicate significance ($p<0.05$), n=3

There were also changes in the total soluble solids (°Brix) in all treated juice samples (Figure 1(b)). In particular, the addition of a high concentration of acidic preservative (CA and AA) would accelerate the reduction of °Brix. The °Brix for juice with 0.8% CA decreased the most, becoming 3.0 lower compared to the initial °Brix on day 0, which is equivalent to a 40.79% reduction compared to control. This was followed by 0.8% AA treatment, which showed a 31.57% reduction against control. The use of certain preservatives, such as 0.1% SA, 0.1% PS, and 0.2% PS gave the smallest declines, to only 0.02 lower compared to the initial °Brix on day 0. As most microorganisms should be inhibited by the added preservatives, the reduction of °Brix is most likely caused by the change in pH caused by the preservatives. This was demonstrated previously in coconut toddy when either 0.015% of sodium metabisulfite, benzoates, or calcium hydroxide was added (Hariharan Singaravadivel, & Alagusundaram, 2014).

Color is also an important determinant of the quality of sugarcane juice. Browning reactions took place in the control juice sample as the L* decreased and a* increased during storage (Table 1). All of the treated juice samples had lighter, greener, and bluer colors than the control samples after a week of storage, indicated by increased L*, decreased a*, and decreased b*. These results demonstrate that the addition of preservatives enhances the color profile of sugarcane juice while inhibiting potential browning reactions (decrease in a*) that might take place after extraction (Monsalve-Gonzalez *et al.*, 1993).

To confirm the findings from the color analysis, an assay to determine the activity of PPO was carried out. Based on Table 2, the PPO enzymatic activity for all treated samples was significantly lower than for the control after 7 days. The biggest reduction in PPO activity (from day 1 to day 7) was achieved by 0.1% PS, although a higher concentration of PS did not increase its efficiency. A similar result was reported by Sanchís *et al.* (2016) and Alaaeldin *et al.* (2016), observing that the most effective used concentration in minimizing the PPO activity was a low concentration of PS (Alaaeldin, Ismail, Shanan, Asa Abo, & Rei 2016; Sanchís *et al.*, 2016). Nevertheless, AA was shown to be the most effective preservative, as both concentrations (0.4% and 0.8%) caused a significant reduction in PPO activity, compared to the control experiment. AA minimizes the activity of PPO enzymatic browning by reducing *o*-quinones back to phenolic compounds before they produce brown pigments (Vámos-Vigyázó & Haard, 1981).

NEBI is also known as the Maillard reaction, and is initiated by the condensation of the carbonyl group of reducing sugars with free amino groups of amino acids. In this study, the NEBI for all juice samples remained almost unchanged throughout the 7 days of storage (Table 2). This observation indicates that the addition of preservatives did not accelerate nor reduced the rate of NEBI. In contrast to PPO, NEBI requires certain amino acids (e.g. lysine) and inducers, such as heat, alkaline conditions, and extreme moisture content to be induced (Croguennec, 2016). Due to the absence

Table 1. The color coordinates of sugarcane juice treated with different preservatives on day 1 and day 7. Day 1 is 24 h after the addition of preservatives. Different superscripts indicate significance within the same column. Data are means of triplicate (n=3) ± SD. Increase in ΔL = sample is lighter, decrease in ΔL = sample is darker; increase in Δa = sample is redder, decrease in Δa= sample is greener; increase in Δb = sample is yellower, decrease in Δb = sample is bluer.

Sample	Day 1			Day 7		
	*L	*a	*b	*L	*a	*b
Control	46.92±2.03^{a}	9.10±0.51^{d}	49.23±2.10^{a}	44.40±2.58^{a}	12.24±0.22^{e}	55.17±5.27^{c}
0.1% SA	46.36±1.49^{a}	11.89±0.18^{e}	54.69±4.65^{b}	47.69±3.13^{ab}	9.22±0.61^{cd}	49.89±4.37^{b}
0.1% SB	46.91±3.49^{a}	11.44±0.47^{e}	54.87±4.81^{b}	46.98±3.02^{a}	8.75±0.24^{c}	48.97±3.48^{b}
0.1% PS	45.65±3.12^{a}	11.85±0.56^{e}	54.41±5.23^{b}	48.03±4.89^{ab}	8.38±0.11^{c}	49.67±3.98^{b}
0.2% SA	47.43±4.37^{a}	11.05±0.43^{e}	54.19±4.83^{b}	49.67±4.28^{b}	9.56±0.58^{d}	49.87±4.16^{b}
0.2% SB	46.98±4.14^{a}	10.87±0.63^{e}	53.74±3.54^{b}	48.13±3.98a^{b}	9.14±0.61^{cd}	50.09±5.03^{b}
0.2% PS	45.38±2.27^{a}	11.52±0.37^{e}	54.11±4.01^{b}	48.73±4.92^{ab}	10.07±0.88^{d}	49.58±6.32^{b}
0.4% AA	54.77±4.12^{b}	7.48±0.14^{c}	54.80±6.42^{b}	54.96±3.99^{c}	4.59±0.04^{a}	43.72±6.14^{a}
0.4% CA	56.50±4.68^{b}	8.05±0.05^{c}	49.53±2.31^{a}	51.74±5.22^{bc}	5.97±0.11^{b}	44.17±3.06^{a}
0.8% AA	65.19±5.71^{c}	3.21±0.06^{a}	45.79±3.39^{a}	56.64±4.66^{c}	3.18±0.03^{a}	41.25±2.71^{a}
0.8% CA	63.62±5.09^{c}	6.09±0.21^{b}	46.20±1.03^{a}	54.27±5.08^{c}	5.27±0.21^{b}	40.17±4.04^{a}

of these factors, the rate of NEBI was low. As mentioned before, AA was able to reduce the enzymatic browning (PPO)significantly. However, AA at all concentrations, failed to halt NEBI. As AA showed amongst the lightest, greenest, and bluest colors, these observations might indicate that the browning in sugarcane juice is primarily contributed by PPO, but not NEBI. It is currently unknown why AA affects PPO and NEBI differently.

3.2 Microbial and toxicological safety of preservatives in red sugarcane juice

Table 3 shows the results for the total plate count (TPC) and yeast and molds in the treated juice samples after 7 days. It was clear that PS, at all concentrations, was the most effective preservative, as it is the only treatment that resulted in not too numerous to count (TNTC) colonies at 10^{-2} dilution in TPC. Furthermore, PS was also able to significantly reduce the yeast and mold count at all concentrations. SB was also shown to be efficient at reducing yeast and mold, but this preservative was not efficient for reducing bacterial growth (high TPC number). All other preservatives were unable or had limited capability to reduce microbial presence at 10^{-2} dilution, which indicates that they are not suitable for use in the red sugarcane juice. The antimicrobial effects of sorbate have been demonstrated by a considerable amount of prior research (Stopforth *et al.*, 2005).

The brine shrimp lethality assay using brine shrimp *naupii* is a useful test for preliminary assessment of the toxicity of the preservatives, via determining the median lethal concentration (LC_{50}). A low LC_{50} indicates increased toxicity of the juice sample. The mortality rates and LC_{50} of the brine shrimp *naupii* were recorded after 24 hours of exposure as shown in Table 4. The results demonstrate that 0.2% PS is the most toxic preservative, while 0.1% SB was the least toxic preservative in the red sugarcane juice. These data are concordant with the microbiological analyses, which showed PS was the most effective preservative in inhibiting microbiological growth. Although the PS showed the highest toxicity, this preservative is still considered safe, as this sample demonstrated LC_{50} > 1000 µg/ml, which is considered non-toxic based on Meyer's toxicity index (Meyer *et al.*, 1982).

4. Conclusions

All the preservatives were shown to be non-toxic and effective in improving the color profile of red sugarcane juice, although they were not able to reduce the rate of NEBI. AA showed an excellent ability to reduce enzymatic browning

Table 2. PPO enzyme and NEBI activities for the samples on day 1 and day 7. Day 1 is 24 h after the addition of preservatives. Different superscripts indicate significance within the same column. Data are means of triplicate (n=3) ± SD.

Sample	PPO enzyme activity ($U.ml^{-1}$)		NEBI	
	Day 1	Day 7	Day 1	Day 7
Control	8.45±0.13^{e}	4.25±0.11^{c}	0.121±0.6^{a}	0.211±0.072^{b}
0.1% SA	6.20±0.65^{d}	2.82±0.08^{b}	0.133±0.033^{a}	0.210±0.126^{ab}
0.1% SB	5.60±0.45^{c}	2.42±0.07^{b}	0.131±0.012^{a}	0.198±0.124^{ab}
0.1% PS	6.22±0.92^{d}	0.94±0.06^{a}	0.123±0.019^{a}	0.207±0.151^{ab}
0.2% SA	6.55±0.97^{d}	2.79±0.13^{b}	0.130±0.028^{a}	0.200±0.128^{ab}
0.2% SB	8.50±0.94^{e}	3.63±0.08^{c}	0.131±0.032^{a}	0.195±0.131^{ab}
0.2% PS	6.00±0.79^{cd}	2.78±0.07^{b}	0.138±0.13^{a}	0.214±0.128^{ab}
0.4% AA	0.38±0.12^{a}	0.32±0.14^{a}	0.126±0.082^{a}	0.204±0.091^{b}
0.4% CA	4.00±0.18^{b}	1.74±0.01^{b}	0.147±0.072^{a}	0.216±0.123^{ab}
0.8% AA	0.14±0.11^{a}	0.12±0.06^{a}	0.146±0.066^{a}	0.218±0.067^{b}
0.8% CA	3.11±0.09^{b}	1.40±0.07^{ab}	0.153±0.118^{a}	0.213±0.081^{b}

Table 3. Total plate count (TPC) of treated juice samples on day 7. TNTC: Too numerous to count, ND: Non-detected

Sample	Day 7			
	TPC (cfu/g)		Yeast and molds (cfu/g)	
	10^{-2} dilution	10^{-3} dilution	10^{-2} dilution	10^{-3} dilution
Control	TNTC	TNTC	TNTC	TNTC
0.1% SA	TNTC	3.01×10^{6}	TNTC	TNTC
0.1% SB	TNTC	TNTC	2.15×10^{5}	8×10^{5}
0.1% PS	1.2×10^{4}	1.5×10^{4}	2.55×10^{4}	1.5×10^{4}
0.2% SA	TNTC	2.28×10^{6}	TNTC	1.96×10^{6}
0.2% SB	TNTC	TNTC	1×10^{3}	5×10^{3}
0.2% PS	2×10^{3}	1×10^{4}	1.5×10^{3}	ND
0.4% AA	TNTC	TNTC	TNTC	TNTC
0.4% CA	TNTC	TNTC	TNTC	TNTC
0.8% AA	TNTC	1.79×10^{6}	TNTC	TNTC
0.8% CA	TNTC	TNTC	TNTC	TNTC

Table 4. The mortality rate of brine shrimp of treated juice samples at different concentrations

Sample	Mortality (%)			LC_{50} (in ppm)	Toxicity ranking
	1,000,000	100,000	10,000		
Control	100	25	15	434,125	11
Negative control	0	0	0	-	-
0.1% SA	100	33.33	17.65	363,625	8
0.1% SB	100	35	15	371,625	10
0.1% PS	100	50	25	238,143	4
0.2% SA	100	30	30	341,286	7
0.2% SB	100	45	20	313,571	6
0.2% PS	100	70	30	64,833	1
0.4% AA	100	45	25	273,857	5
0.4% CA	100	30	20	368,125	9
0.8% AA	100	50	35	185,333	3
0.8% CA	100	45	40	180,667	2

but was not very efficient in reducing microbial growth. In contrast, PS was able to reduce microbial growth with a good ability to reduce enzymatic and non-enzymatic browning, indicating that PS might be the best preservative for red sugarcane juice.

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