

Effect of pasteurization treatment and calamansi (*Fortunella japonica*) juice on the physicochemical, microbiological, and sensory characteristics of black stem sugarcane juice

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Abstract

Sugarcane juice deteriorates rapidly as enzymatic browning and microbial spoilage take place soon after juice extraction. Therefore, the effects of pasteurization treatment (70°C for 10 minutes) and addition of calamansi juice at different concentrations (0, 1.0, 1.5, and 3.0%, v/v) on the physicochemical (color, pH, total soluble solid, titratable acidity, and peroxidase enzymatic activity), microbiological (total plate count, yeast and mold), and sensory characteristics of black stem sugarcane juice were investigated. Quantitative descriptive analysis (QDA[®]) was conducted by eight trained panelists to determine the intensity of the sensory attributes of sugarcane juice, such as brownish color, grassy aroma, citrus aroma, sweetness, sourness, sweet aftertaste, and overall acceptability using a 15 cm unstructured line scale. The results showed that the pasteurization treatment significantly ($p < 0.05$) increased the L^* value (lightness) of sugarcane juice at 0% (v/v) of calamansi juice. Increasing the concentration of calamansi juice decreased the pH value and increased the titratable acidity of the sugarcane juice at 5% significance level. The relative effectiveness in reducing the peroxidase (POD) enzymatic activity and microbial load of sugarcane juice was shown after the addition of calamansi juice and pasteurization treatment, respectively. Significant ($p < 0.05$) changes in the intensity of brownish color, grassy aroma, and sweet aftertaste of sugarcane juice were observed after the pasteurization treatment at 0% (v/v) calamansi juice; whereas, the addition of calamansi juice at different concentrations increased the intensity of citrus aroma and sourness, but decreased the intensity of grassy aroma and sweet aftertaste of sugarcane juice at 5% significance level. Hence, pasteurization treatment coupled with the addition of 1.5% (v/v) calamansi juice was effective in achieving microbial stability and consumer's acceptability on sugarcane juice.

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Introduction

Sugarcane (*Saccharum officinarum* sp.) has been planted in Malaysia since the 19th century for sugar and juice production (Tan, 1989). It is a giant grass, which belongs to family Poaceae. The color of its stems varies from pale green to green, red to purple and dark purple to almost black (James, 2004). The extracted juice from sugarcane contains water (80.00 – 81.70%), crude fiber (13.24 – 16.62%), and ash (0.28 – 0.48%). It is a nutritious drink with an appreciably high amount of minerals, such as calcium, phosphorus, and iron (Chauhan *et al.*, 2002). The flavonoids, phenolic compounds, and chlorophyll found in sugarcane juice are expected to provide antioxidant protection to humans (Kadam *et al.*, 2008).

Although sugarcane juice is a popular thirst-quenching drink with plenty of health benefits, the marketing of sugarcane juice is limited by its short

shelf life. The color of sugarcane juice becomes less appealing when the color turns from greenish to brownish due to the formation of melanin pigments soon after juice extraction. The color change is due to the oxidation of the phenolic compounds by the peroxidase (POD) and polyphenol oxidase (PPO) enzymes in the sugarcane juice, which is termed as enzymatic browning (Qudsieh *et al.*, 2002). The enzymatic browning effect could be minimized through enzyme inactivation by heat treatment and the addition of ascorbic acid (Javdani *et al.*, 2013; Kunitake *et al.*, 2014). However, POD is reported to be more thermal resistant compared to PPO, as POD enzymatic activity has still been detectable in sugarcane juice at 100°C (Buchelin and Robinson, 1994).

The properties of sugarcane juice with low acidity, high water activity, and high sugar content (Yusof *et al.*, 2000) make it prone to rapid deterioration by microbial spoilage. The presence of lactic acid

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bacteria, especially *Leuconostoc* sp., which is naturally found in sugarcane juice, utilize the sucrose in the juice, and, subsequently produce dextran, ethanol, and organic acid (Frazier and Westhoff, 1998). Improper cleaning of sugarcane and poor sanitation conditions during juice extraction have increased the risk of microbial contamination (Ali *et al.*, 2015). Therefore, pasteurization treatment with High-Temperature Short Time (HTST) is widely used in juice processing to destroy viable microorganisms, including *Leuconostoc* sp., without much affecting on sensory quality of the juice (Eggleston, 2002). Most preservative attempts to prolong the shelf life of sugarcane juice have focused on pasteurization treatment, incorporating citrus juice to reduce the pH below 4.6, and applying preservatives such as sulfur dioxide (Bhupinder *et al.*, 1991; Yusof *et al.*, 2000).

In the present research, calamansi (*Fortunella japonica*) juice, which is highly acidic (pH 2.4) (Lee, 2000) and contains an appreciably high amount of citric acid (5.52%) (Morton, 2013), is used as an acidifying agent and antioxidant to reduce the pH and minimize the oxidation of the phenolic compounds in sugarcane juice, respectively. Moreover, the blending of calamansi juice, which has a citrus flavor, is believed to improve the sensation of sugarcane juice. Therefore, the effect of pasteurization treatment and different concentrations of calamansi juice on the physicochemical, microbiological, and sensory characteristics of sugarcane juice are investigated.

Materials and Methods

Materials

Black stem sugarcanes were obtained from Merbok, Sungai Petani (Kedah, Malaysia). Calamansi fruits and commercial sugarcane beverage (in Tetra Pak packaging) (Yeo's brand) were purchased from Tesco hypermarket, (Penang, Malaysia). Sodium hydroxide (NaOH), sodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), hydrogen peroxide (H_2O_2), and guaiacol were obtained from Sigma-Aldrich (Germany). Peptone powder, plate count agar, and potato dextrose agar were obtained from Merck (Germany).

Sample preparation

Sugarcanes were cleaned and washed with tap water to remove any foreign material, mud or dirt presented on the surface of the stem. After washing, a roller power crusher was used to extract the juice. Then, the sugarcane juice obtained was collected and filtered using a muslin cloth. Filtered calamansi juice was added into the sugarcane juice at different

concentrations (0, 1.0, 1.5 and 3.0%, v/v) and divided into 2 batches (with and without pasteurization treatment). Pasteurization was carried out at 70°C for 10 minutes (Chauhan *et al.*, 2002) by immersing the glass bottle (containing sugarcane juice) in a water bath (Mermmert, Germany) with temperature control. After 10 minutes, glass bottles were taken from the water bath and immediately immersed in an ice water to drop the temperature around 25°C.

Experimental design

A completed randomized design, with a 2×4 factorial set of pasteurization treatment (with and without) and the addition of calamansi juice at different concentration (0, 1.0, 1.5 and 3.0%, v/v), was adopted.

Determination of color, pH, titratable acidity (TA), and total soluble solid (TSS)

The color of sugarcane juice was measured using a spectrophotometer (Minolta Spectrophotometer CM-3500d, Japan). The results of color obtained were expressed in lightness (L^*), redness ($+a^*$), and yellowness ($+b^*$) (Tan *et al.*, 2014). The pH of the juice was measured using a pH meter (Mettler Toledo Seven Easy pH meter S20-K, Columbus) at 25°C (Tan *et al.*, 2014). Titratable acidity was determined by titrating a mixture of 10 mL of juice in 90 mL of distilled water with 0.1 N NaOH to an endpoint of pH 8.2 (Kunitake *et al.*, 2014). The titratable acidity of juice was expressed as a percentage of citric acid according to equation 1.

$$\text{Titratable acidity (\% of citric acid)} = \frac{(v \times N \times M \times 100 \text{ mL})}{W} \quad (1)$$

where V is volume of NaOH (mL); N is normality of NaOH; M is equivalent weight of citric acid (0.064 mg / mEq); W is weight of sample (mL).

Total soluble solid of the juice was measured using a digital refractometer (Hanna Instruments 96801 Digital Refractometer, USA) at 25 °C and expressed in °Brix (Tan *et al.*, 2014). The measurements for color, pH, titratable acidity (TA), and total soluble solid (TSS) were performed in triplicate.

Determination of peroxidase (POD) enzymatic activity

The peroxidase (POD) enzymatic activity of sugarcane juice was determined according to the method described by Freire *et al.* (2015). A total amount of 0.6 mL of sugarcane juice was mixed with 2 mL of sodium phosphate buffer (pH 6.00). The mixture was kept in a closed universal bottle

(30 mL) with a cap and left at 25°C for 1.5 minutes. Approximately 0.2 mL of hydrogen peroxide and 0.2 mL of guaiacol were then added to the mixture. The absorbance was measured at 470 nm using a UV-vis spectrophotometer (UV-vis spectrophotometer – 1650 PC, Shimadzu Europe). The absorbance reading was taken every 30 seconds for 2 minutes. A blank solution was prepared by mixing 2 mL of sodium phosphate buffer, 0.2 mL of hydrogen peroxide, and 0.2 mL of guaiacol. The POD enzymatic activity was expressed in U/mL with one unit equivalent to a variation of 0.001 absorbance per minute per mL of sugarcane juice using equation 2 (Kunitake *et al.*, 2014).

$$\text{POD activity (U/ml)} = (Ab_{\text{sample}} - Ab_{\text{blank}}) / (0.001 \times V) \quad (2)$$

where Ab_{sample} is the sample absorbance; Ab_{blank} is the blank absorbance; and V is the volume (mL) of sample.

Microbiological analysis

The determination of microbial count was conducted according to the standard method of FDA's Bacteriological Analytical Manual (BAM), 1998. The pour plate method was used for determination of the total plate count (TPC) as well as the yeast and mold count. A serial dilution (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5}) of the juice was made and aliquots of 1 mL were added to a petri dish. For TPC, approximately 15 mL of molten plate count agar (PCA) was then added to each of the petri dishes and incubated at 37°C for 48 hours in an incubator (Ambient-High Temperature Incubator Model Memmer, Germany). In addition, 15 ml of molten potato dextrose agar (PDA) was added to petri dish containing 1 mL of diluted sample for determination of the yeast and mold count by incubating it at $25 \pm 1^\circ\text{C}$ for 72 hours. All the results were expressed as colony forming units per mL sample (CFU/mL) using the equation 3.

$$N \text{ (CFU/mL)} = \frac{\Sigma C}{[(1 \times n1) + (0.1 \times n2) \times d]} \quad (3)$$

where, N is number of Colony Forming Unit per mL; ΣC is sum of all colonies on all plate counted; $n1$ is number of plates in the first dilution counted; $n2$ is number of plates in the second dilution counted; and d is dilution from which the first counts were obtained.

Quantitative descriptive analysis (QDA®)

Eight panelists (3 male and 5 female) aged 23 to 24

years old, who are students of the School of Industrial Technology, Universiti Sains Malaysia, were selected and involved in the quantitative descriptive analysis (QDA®). Candidates were preliminarily selected based on their interest and willingness. Then, a screening session was conducted to determine the capability of the candidate to recognize and detect the sourness (0.05% citric acid) and sweetness (1.0% sucrose) versus plain water using triangle test. Those candidates who were able to get correct answer were selected as qualified panelists (Zoecklein, 2012).

Panelists were trained for 6 sessions, 2 hours per session. The Quantitative descriptive analysis (QDA®) method and Spectrum TM Descriptive Analysis method (Meilgaard *et al.*, 2006) were used to train the panelists and evaluate the sample. Panelists were asked to generate terminology for the sensory attributes based on the sugarcane juice produced and a commercial sugarcane beverage (in Tetra Pak packaging). Six sensory attributes were generated with the consensus of all panelists. These comprised brownish color, grassy aroma, citrus aroma, sweetness, sourness, and sweet aftertaste. The reference standards and their intensities for sweetness and sourness attributes were based on the Spectrum Intensity Scales for Descriptive Analysis (Meilgaard *et al.*, 2006), while, the reference standards and their intensities for other sensory attributes (brownish color, grassy aroma, citrus aroma, and sweet aftertaste) were generated by the panelists. The description, reference, and intensity of each sensory attribute are shown in Table 1. For the following training session, all panelists were trained to familiarize themselves with the reference samples until they consistently marked the intensity for each sensory attribute.

For the sensory evaluation, sugarcane juice with and without pasteurization treatment at different concentrations of calamansi juice was served to panelists at 25°C in paper cups coded with three-digit random numbers. The juice was served randomly to each of the panelists together with a set of reference samples. Plain water was provided to the panelists to rinse their mouth before and between testing of the juice. The panelists evaluated the juice in an individual booth in the sensory room, School of Industrial Technology, USM. The panelists were asked to mark the intensity of the sensory attributes for each of the juice using 15 cm unstructured line scales. Each of the juice was evaluated in duplicate on the separate testing day.

Statistical analysis

All the results were expressed as mean \pm

Table 1. Description and intensity of reference materials

Attribute	Description	Reference	Intensity (cm)
Color			
Brownish	Intensity of sample color	Color paint: Jasmine 1129, Marigold 1328, Barbary gold 1329	2.2, 7.7, 13.2
Aroma			
Grassy	Aromatics of grass	Commercial sugar cane beverage (Yeo's brand), wild grass	0.8, 13.7
Citrus	Aromatics of citrus	1%, 5% of calamansi juice	2.7, 12.4
Taste			
Sweetness	Basic taste sensation elicited by sucrose	^a 5%, 10% of sucrose solution	5.0, 10.0
Sourness	Basic taste sensation elicited by citric acid	^a 0.08%, 0.15% of citric acid	5.0, 10.0
Sweet aftertaste	Taste of sweetness detected after swallow the sample	Unpasteurized sugar cane juice with adding of 1.5% calamansi juice	9.4

^a Adapted from (Meilgaard *et al.*, 2006). Intensity of each attribute for each reference sample was calculated as the mean rating of the group of eight panelists using a 15 cm unstructured line scale where 0 = none and 15 = the highest intensity

standard deviation (SD). One-way analysis of variance (ANOVA) and independent sample t-test were used for analyzing the data. Statistical Package for Social Science (SPSS) Software version 22 (IBM Corporation, New York, USA) was used to perform the statistical analysis.

Results and Discussion

Color

The effects of pasteurization treatment and different concentrations of calamansi juice on the color of sugarcane juice are shown in Table 2. Pasteurization treatment resulted in a significant ($p < 0.05$) increase in the L^* value (lightness) of sugarcane juice at 0% (v/v) of calamansi juice. This result indicated a lighter color and less browning effect on pasteurized sugarcane juice due to the reduction of polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activity by the heat treatment. A similar result was reported by Kunitake *et al.* (2014); thereby implying the inactivation and denaturation of PPO and POD enzymes to a certain extent depending on the pasteurization parameter. A significant ($p < 0.05$) increase in the L^* value was observed by adding calamansi juice at 1.0% (v/v) and 1.5% (v/v) for unpasteurized and pasteurized sugarcane juice, respectively. This suggested that the antioxidant compounds in the calamansi juice had bound with oxygen and prevented oxidation of the phenolic compounds in the sugarcane juice (Mao *et al.*, 2007). The lower oxygen availability in sugarcane juice reduced both PPO (oxygen-dependent reaction) and POD enzymatic activity (peroxide-dependent reaction) (Queiroz *et al.*, 2008). In addition, the complex forming between the citric acid in the calamansi juice and the copper ion in PPO active site reduced the enzymatic browning reaction of

sugarcane juice (Kunitake *et al.*, 2014).

A significant ($p < 0.05$) increase in the a^* value (redness) was observed after the addition of calamansi juice up to 3.0% (v/v) for both unpasteurized and pasteurized sugarcane juice. Whereas, a significant ($p < 0.05$) increase in the b^* value (yellowness) was shown by adding calamansi juice at 3.0% (v/v) and 1.0% (v/v) for unpasteurized and pasteurized sugarcane juice, respectively. However, the pasteurization treatment caused no significant ($p > 0.05$) change in both the a^* and b^* value of sugarcane juice, which is in accordance with Kunitake *et al.* (2014).

pH, titratable acidity (TA), and total soluble solid (TSS)

The effects of pasteurization treatment and different concentrations of calamansi juice on the pH, titratable acidity (TA), and total soluble solid (TSS) of sugarcane juice are shown in Table 2. No significant ($p > 0.05$) effect was reported on the pH, TA, and TSS of sugarcane juice at 0% (v/v) calamansi juice after pasteurization treatment.

Increasing the concentration of calamansi juice resulted in a decrease in pH and an increase of titratable acidity (TA) in both unpasteurized and pasteurized sugarcane juice at 5% significance level. The similar trend was reported in the previous studies by adding acidic juice, such as anola juice (Sangeeta *et al.*, 2013) and lemon juice (Bhupinder *et al.*, 1991). The addition of calamansi juice at 1.0% (v/v) successfully reduced the pH below 4.6 for both unpasteurized and pasteurized sugarcane juice due to the acidic nature of calamansi juice with a pH of 2.4 (Lee, 2000). This is an important step in turning sugarcane juice (pH 5.02) into an acidified juice, which favors enzymatic and microbiological stability as well as permits the application of pasteurization

Table 2. Effect of pasteurization treatment and different concentration of calamansi juice on color, pH, titratable acidity (TA), and total soluble solid (TSS) of sugarcane juice

Parameter	Treatment	Concentration of calamansi juice (%)			
		0	1.0	1.5	3.0
L*	Unpasteurization	26.23 ± 0.43 ^{Aa}	29.05 ± 1.14 ^{Ab}	29.50 ± 1.18 ^{Ab}	30.38 ± 0.77 ^{Ab}
	Pasteurization	28.55 ± 0.94 ^{Ba}	30.19 ± 0.88 ^{Aab}	30.93 ± 1.06 ^{Ab}	31.50 ± 1.26 ^{Ab}
a*	Unpasteurization	11.16 ± 1.07 ^{Aa}	11.35 ± 0.61 ^{Aa}	12.18 ± 0.25 ^{Aab}	12.85 ± 0.68 ^{Ab}
	Pasteurization	11.43 ± 0.63 ^{Aa}	11.91 ± 0.54 ^{Aab}	12.38 ± 0.86 ^{Aab}	13.01 ± 0.61 ^{Ab}
b*	Unpasteurization	43.83 ± 0.95 ^{Aa}	44.86 ± 1.05 ^{Aab}	45.16 ± 1.07 ^{Aab}	46.06 ± 0.34 ^{Ab}
	Pasteurization	44.31 ± 0.21 ^{Aa}	45.74 ± 0.45 ^{Ab}	46.24 ± 0.31 ^{Ab}	46.97 ± 0.05 ^{Bc}
pH	Unpasteurization	5.02 ± 0.06 ^{Ad}	4.42 ± 0.08 ^{Ac}	4.27 ± 0.05 ^{Ab}	3.95 ± 0.07 ^{Aa}
	Pasteurization	5.15 ± 0.08 ^{Ad}	4.52 ± 0.08 ^{Ac}	4.35 ± 0.05 ^{Ab}	4.05 ± 0.05 ^{Aa}
Titratable acidity (% citric acid)	Unpasteurization	0.07 ± 0.01 ^{Aa}	0.12 ± 0.00 ^{Bd}	0.17 ± 0.00 ^{Bc}	0.23 ± 0.00 ^{Bd}
	Pasteurization	0.07 ± 0.00 ^{Aa}	0.11 ± 0.00 ^{Ab}	0.16 ± 0.00 ^{Ac}	0.20 ± 0.00 ^{Ad}
Total soluble solid (° Brix)	Unpasteurization	17.50 ± 0.40 ^{Aa}	17.40 ± 0.40 ^{Aa}	17.40 ± 0.40 ^{Aa}	17.30 ± 0.40 ^{Aa}
	Pasteurization	17.40 ± 0.50 ^{Aa}	17.30 ± 0.40 ^{Aa}	17.20 ± 0.40 ^{Aa}	17.10 ± 0.40 ^{Aa}

^{A-B} Mean ± standard deviation (n=3) followed by same letters do not differ significantly (p > 0.05) between treatments of sugarcane juice at each concentration of calamansi juice.

^{a-d} Mean ± standard deviation (n=3) followed by same letters do not differ significantly (p > 0.05) between concentrations of calamansi juice in sugarcane juice at each treatment.

Table 3. Effect of pasteurization treatment and different concentration of calamansi juice on Peroxidase (POD) enzymatic activity in sugarcane juice

Parameter	Treatment	Concentration of calamansi juice (%)			
		0	1.0	1.5	3.0
Peroxidase (POD) enzymatic activity (U/mL)	Unpasteurization	749.16 ± 15.32 ^{Ac}	482.50 ± 8.24 ^{Ab}	425.84 ± 14.14 ^{Ab}	316.67 ± 23.57 ^{Aa}
	Pasteurization	656.67 ± 30.64 ^{Ac}	446.67 ± 9.40 ^{Ab}	416.67 ± 21.21 ^{Ab}	296.67 ± 14.14 ^{Aa}

^A Mean ± standard deviation (n=3) followed by same letters do not differ significantly (p > 0.05) between treatments of sugarcane juice at each concentration of calamansi juice.

^{a-c} Mean ± standard deviation (n=3) followed by same letters do not differ significantly (p > 0.05) between concentrations of calamansi juice in sugarcane juice at each treatment.

treatment (Kunitake *et al.*, 2014). The total soluble solid (TSS) of all the sugarcane juice studied was found to range from 17.1 to 17.5°Brix. No significant (p > 0.05) difference was observed in the TSS value with increasing concentration of calamansi juice. The similar TSS trend was observed in the previous study as increasing the proportion of anola juice in sugarcane juice (Sangeeta *et al.*, 2013).

Peroxidase (POD) enzymatic activity

Polyphenol oxidase (PPO) and peroxidase (POD) are the main enzymes involved in the browning of sugarcane juice after the extraction process. These enzymes cause oxidation of the phenolic compounds in sugarcane juice to form chemically reactive quinones, which is then polymerized to form a melanin pigment (Kunitake *et al.*, 2014). Kunitake *et al.* (2014) claimed that POD was more heat resistant compared to PPO, where enzyme inactivation of POD and PPO was achieved at a pasteurization temperature of 95°C for 10 seconds and 85°C for 10 seconds, respectively. Therefore, the successful inactivation of POD could also indirectly inhibit the PPO enzymatic activity.

The effects of pasteurization treatment and

different concentrations of calamansi juice on the peroxidase (POD) enzymatic activity of sugarcane juice are shown in Table 3 with a wide variation ranging from 296.67 to 749.16 U/mL. This result is fairly consistent with Marques *et al.* (2013), who reported a POD enzymatic activity of 600 – 750 U/mL for fresh extracted sugarcane juice. However, lower POD enzymatic activity in sugarcane juice was reported by Kunitake *et al.* (2014), which is probably due to the differences in variety, maturity, harvesting period, and composition (sugar content, salt content, pH, quantity and availability of phenolic compound) of sugarcane (Tan *et al.*, 2014).

The result showed that pasteurization treatment caused no significant (p > 0.05) reduction on the peroxidase (POD) enzymatic activity of sugarcane juice at each concentration of calamansi juice. This result suggested that pasteurization treatment (70°C for 10 min) used was ineffective to inactivate POD enzymatic activity due to the nature of high thermal resistance.

With increasing concentration of calamansi juice, a decreasing (p < 0.05) trend in the peroxidase (POD) enzymatic activity was observed for both unpasteurized and pasteurized sugarcane juice. This

Table 4. Effect of pasteurization treatment and different concentration of calamansi juice on growth of microorganism in sugarcane juice

Parameter	Treatment	Concentration of calamansi juice (%)			
		0	1.0	1.5	3.0
Total plate count (CFU/mL)	Unpasteurization	3.95×10^7	2.51×10^7	1.94×10^7	1.75×10^7
	Pasteurization	4.73×10^3	2.41×10^3	2.19×10^3	1.81×10^3
Yeast and mold (CFU/mL)	Unpasteurization	4.15×10^5	2.95×10^5	2.04×10^5	1.25×10^5
	Pasteurization	6.27×10^2	6.05×10^2	5.27×10^2	4.27×10^2

result suggested that an increase of POD enzyme inactivation extent as increasing the acidity of sugarcane juice. This is probably due to the decrease of POD enzymatic stability by the pH reduction and the protective action of calamansi juice (Kunitake *et al.*, 2014). Calamansi juice acts as a reducing agent, which actively binds to oxygen to lower the oxygen availability for the enzymatic browning reaction of sugarcane juice (Mao *et al.*, 2007). The reduction of enzymatic browning effect may be attributed to the citric acid in calamansi juice, as it will form a complex with the copper ion present in the PPO active site (Kunitake *et al.*, 2014).

Microbiological analysis

The effects of pasteurization treatment and different concentrations of calamansi juice on the microbial load of sugarcane juice are shown in Table 4. A heavy microbial load was found in the sugarcane juice studied, ranging from 1.81×10^3 - 3.95×10^7 CFU/mL for the total plate count (TPC), with a maximum viable count on unpasteurized sugarcane juice with 0% (v/v) calamansi juice. This result is in accordance with Ali *et al.* (2015), who reported 3.0×10^7 CFU/mL for the TPC in fresh sugarcane juice sold at Peshawar City, Khyber Pakhtunkhwa, Pakistan.

By increasing concentration of calamansi juice, the total viable microbial load of sugarcane juice was decreased, which suggested the suppression of microbial growth in the acidic condition as most of the microorganisms grow well at a pH value of around 7.0 (Kunitake *et al.*, 2014). However, a viable microbial count was still observed in sugarcane juice even though the pH was lower than 4.6 (Table 2). This situation could be explained by the presence of acid tolerant microorganisms, such as lactic acid bacteria (*Leuconostoc* sp.) with the ability to thrive well in acidic conditions (Battcock, 1998).

The yeast and mold count was reported to be in the range of 4.27×10^2 - 4.15×10^5 CFU/mL. The highest (4.15×10^5 CFU/mL) count was found in unpasteurized sugarcane juice with 0% (v/v) calamansi juice, which is fairly consistent with Ali *et al.* (2015) with a finding of 4.00×10^5 CFU/mL

of yeast and mold in fresh sugarcane juice sold in Pakistan. Increasing the concentration of calamansi juice reduced the yeast and mold count, due to the acidic condition created in the sugarcane juice, which retarded and restricted the growth of yeast and mold (Sangeeta *et al.*, 2013).

The pasteurization treatment of sugarcane juice achieved a 4 log reduction and 3 log reduction in the total plate count (TPC), and yeast and mold count, respectively. The considerable reduction in the microbial count suggested the relative effectiveness of thermal treatment in destroying microorganisms compared to the acidification treatment through the addition of calamansi juice. This result was supported by Eggleston (2002), who claimed that boiling could destroy or vastly reduce the acid resistant *Leuconostoc* sp. bacteria, which is normally found in sugarcane juice. Furthermore, the combined treatment of pasteurization and the addition of 3.0% (v/v) of calamansi juice showed the lowest count for both the TPC, and the yeast and mold count.

Quantitative descriptive analysis (QDA®)

The effects of pasteurization treatment and different concentrations of calamansi juice on the sensory characteristics of sugarcane juice are shown in Table 5. Pasteurization treatment significantly ($p < 0.05$) reduced the intensity of brownish color in sugarcane juice at 0% (v/v) calamansi juice. As the increasing concentration of calamansi juice, the intensity of brownish color decreased significantly ($p < 0.05$) in both unpasteurized and pasteurized sugarcane juice. This result is attributed to the reduction of enzymatic browning activity through enzyme inactivation and denaturation by the thermal treatment as well as the protection effect of antioxidant from calamansi juice. Thermal degradation on pasteurized sugarcane juice, such as chlorophyll degradation, Maillard reaction, and caramelization may contribute to the color alteration on sugarcane juice as well (Tomasik, 1989).

The grassy aroma in sugarcane juice is recognized as being one of the important flavors that are directly linked to the freshness of the juice. Pasteurization treatment significantly ($p < 0.05$) reduced the

Table 5. Effect of pasteurization and different concentration of calamansi juice on sensory characteristics of sugarcane juice

Sensory attribute	Treatment	Concentration of calamansi juice (%)			
		0	1.0	1.5	3.0
Browish color	Unpasteurization	9.7 ± 0.3 ^{Bd}	6.4 ± 0.4 ^{Ac}	4.6 ± 0.4 ^{Ab}	2.7 ± 0.1 ^{Aa}
	Pasteurization	7.2 ± 0.4 ^{Ac}	6.1 ± 0.6 ^{Ac}	3.6 ± 0.5 ^{Ab}	2.3 ± 0.2 ^{Aa}
Grassy aroma	Unpasteurization	11.4 ± 0.5 ^{Bc}	10.3 ± 0.3 ^{Bb}	9.5 ± 0.4 ^{Bb}	7.8 ± 0.1 ^{Ba}
	Pasteurization	5.2 ± 0.2 ^{Ac}	4.1 ± 0.1 ^{Ab}	4.2 ± 0.1 ^{Ab}	3.0 ± 0.2 ^{Aa}
Citrus aroma	Unpasteurization	1.1 ± 0.2 ^{Aa}	3.1 ± 0.2 ^{Bb}	4.8 ± 0.3 ^{Bc}	7.4 ± 0.2 ^{Bd}
	Pasteurization	0.5 ± 0.1 ^{Aa}	1.0 ± 0.1 ^{Ab}	2.3 ± 0.2 ^{Ac}	3.0 ± 0.1 ^{Ad}
Sweetness	Unpasteurization	13.1 ± 0.3 ^{Ac}	12.2 ± 0.4 ^{Ac}	11.0 ± 0.3 ^{Bb}	10.0 ± 0.1 ^{Ba}
	Pasteurization	12.2 ± 0.2 ^{Ad}	10.9 ± 0.2 ^{Ac}	9.6 ± 0.2 ^{Ab}	8.4 ± 0.1 ^{Aa}
Sourness	Unpasteurization	0.5 ± 0.1 ^{Aa}	1.5 ± 0.3 ^{Ab}	2.4 ± 0.3 ^{Ac}	4.6 ± 0.1 ^{Ad}
	Pasteurization	0.4 ± 0.1 ^{Aa}	1.3 ± 0.1 ^{Ab}	2.3 ± 0.3 ^{Ac}	4.3 ± 0.2 ^{Ad}
Sweet aftertaste	Unpasteurization	12.2 ± 0.1 ^{Ba}	10.9 ± 0.6 ^{Ab}	9.2 ± 0.1 ^{Ac}	7.0 ± 0.5 ^{Ad}
	Pasteurization	10.0 ± 0.1 ^{Aa}	9.3 ± 0.4 ^{Ab}	8.0 ± 0.2 ^{Ac}	6.1 ± 0.3 ^{Ad}
Overall acceptability	Unpasteurization	8.7 ± 0.5 ^{Ba}	11.4 ± 0.4 ^{Bb}	12.4 ± 0.3 ^{Bb}	8.9 ± 0.5 ^{Ba}
	Pasteurization	6.4 ± 0.2 ^{Aa}	7.5 ± 0.2 ^{Ab}	8.1 ± 0.2 ^{Ac}	7.0 ± 0.1 ^{Ad}

^{A-B} Mean ± standard deviation (n=3) followed by same letters do not differ significantly ($p > 0.05$) between treatments of sugarcane juice at each concentration of calamansi juice.

^{a-d} Mean ± standard deviation (n=3) followed by same letters do not differ significantly ($p > 0.05$) between concentrations of calamansi juice in sugarcane juice at each treatment.

intensity of the grassy aroma of sugarcane juice at 0% (v/v) calamansi juice. This is probably due to the evaporation of the heat sensitive flavor compounds in the sugarcane juice, which resulted in the lower intensity of grassy aroma detected in the pasteurized sugarcane juice. The addition of 1.0% (v/v) calamansi juice caused a decrease in the intensity of grassy aroma; whereas, an increase in the intensity of citrus aroma at 5% significance level for both unpasteurized and pasteurized sugarcane juice. This situation suggested the dominant effect of the citrus aroma from calamansi juice due to the presence of volatile components, which contributed to the citrusy and woody note aroma (Barboni *et al.*, 2009).

The pasteurization treatment caused no significant ($p > 0.05$) effect on the sweetness and sourness attribute of sugarcane juice at 0% (v/v) calamansi juice (Table 5). The sourness of both unpasteurized and pasteurized sugarcane juice increased significantly ($p < 0.05$) as increasing the concentration of calamansi juice. This is due to the production of excessive hydrogen ions from the organic acid present in calamansi juice. Meanwhile, the addition of calamansi juice up to 1.5% (v/v) significantly ($p < 0.05$) reduced the sweetness in both unpasteurized and pasteurized sugarcane juice. The result showed that the sweetness and sourness of sugarcane juice were influenced by the TSS/TA ratio (Shahidi *et al.*, 2004).

The aftertaste is described as the taste that is perceived immediately after a food product is swallowed (Meilgaard *et al.*, 2006). In this study, the sweet aftertaste of sugarcane juice was recognized as being dominant by the panelists. The pasteurization

treatment significantly ($p < 0.05$) decreased the intensity of the sweet aftertaste of sugarcane juice at 0% (v/v) calamansi juice (Table 5). With increasing concentration of calamansi juice, the intensity of the sweet aftertaste was significantly ($p < 0.05$) decreased for both unpasteurized and pasteurized sugarcane juice. The reduction of the sweet aftertaste is probably due to the dominance of sour taste and citrus aroma in sugarcane juice after adding the calamansi juice.

The overall acceptability of sugarcane juice reduced significantly ($p < 0.05$) at each concentration of calamansi juice after pasteurization treatment. This is caused by the detrimental thermal effect on sensory quality of sugarcane juice especially the loss of grassy aroma. Significant ($p < 0.05$) higher score of overall acceptability was shown by sugarcane juice with 1.0% and 1.5% (v/v) calamansi juice among unpasteurized batch; whereas, sugarcane juice with 1.5% (v/v) calamansi juice obtained the significant ($p < 0.05$) higher score among pasteurized batch. This result suggested the addition of calamansi juice at 1.0 - 1.5% (v/v) may improve the sensory characteristics of sugarcane juice by imparting refreshing sensation without over-masking the grassy aroma.

Conclusion

The addition of calamansi juice significantly ($p < 0.05$) increased the L^* value (lightness) of sugarcane juice, corresponding to the decrease of browning effect. In reducing the peroxidase (POD) enzymatic activity, calamansi juice was shown to be more effective compared to pasteurization treatment; whereas, pasteurization treatment was effective in

ensuring the safety of sugarcane juice by achieving a 4 log reduction in the microbial load.

The pasteurization treatment significantly ($p < 0.05$) reduced the overall acceptability of sugarcane juice due to the degradation of the sensory quality by the heat treatment especially evaporation of volatile aromatic compounds in sugarcane juice. However, the addition of calamansi juice at 1.0 – 1.5% (v/v) in sugarcane juice has improved the consumer's acceptability. Hence, the combination of pasteurization treatment and the addition of calamansi juice at 1.5% (v/v) on sugarcane juice is considered as an effective treatment in assuring product safety, stability, and good quality.

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