Physicochemical and sensory characteristic of treated sugarcane juice

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Abstract. Sugarcane juice contains high sugar content which can deteriorate rapidly after the juice extraction process. In large juice industry, the deterioration of sugarcane juice can be reduced by pasteurisation and sterilisation treatment process. However, preservation techniques impart undesirable effect on sugarcane juice in terms of taste, colour, appearance and acceptability of the consumer. Therefore, in this study, the effect of pasteurisation process and addition of Spirulina platensis (S. platensis) water extract (0.2% vol/vol) were investigated for its physicochemical (pH, total soluble solid, colour and turbidity), microbiological evaluation (total plate count) and sensory characteristic of sugarcane juice. The addition of S. platensis water extract in sugarcane juice lower the pH value of tested sample contributing to the increase in the stability of sugarcane juice. The other physicochemical tests (total soluble solid, colour and turbidity) have definite correlation with the changes of pH value but not significant (p>0.05) in term of addition of S. platensis water extract into sugarcane juice samples. The microbiological evaluation revealed that high number of colony presence in sugarcane juice after storage for about two weeks and their number were reduced until end of testing period which may result from the depletion of nutrient and inhibition of by-product of reactions that happen in sugarcane juice sample. In terms of sensory testing, the addition of S. platensis water extract in sugarcane juice scored best as compared to control sample even though it does not give significant value (p>0.05) compared to control sample. In conclusion, the addition of S. platensis water extract for treated sugarcane juice is significant as it improves consumer acceptability. The water extract could also acts as colouring agent in sugarcane juice without affecting the physicochemical and sensory properties of sugarcane juice.

1. Introduction
Since ancient times, the sugarcane plants (Saccharum officinarum, L) were cultivated in Asia. The introduction of this plant to the New World was made by Columbus in 1493 and henceforth become one of the important cash crops worldwide [1]. The plant was discovered by Linnaeus in 1753 in his book Species Plantarum in which the generic name of was derived from Greek word, Sacharon which mean sugar [2]. The sugarcane plant can be widely found in Malaysia and among the vast variety of sugarcane plant in Malaysia, the yellow stem variety known as Tebu Kuning is the most popular choice of making fresh sugar cane juice [3].
Sugarcane juice has low pH (about 4.6) and consist of 20% of total soluble solid. Most significant dissolved solids that exist within the juice are saccharose (17%), glucose (0.4%), fructose (0.2%) and nitrogenous substances [4]. Due to its refreshing and sweetness flavour, sugarcane juice often functions as thirst appetiser in many South East Asia countries [5]. Since the sugar content in sugarcane juice is quite high (between 15-18%), studies by Tee et al., (1997), Easa, (2000), and Yusof et al., (2000) as cited in Lo et al., (2007) suggested to consume it as natural energy drink [5]. However, high content of sugar compound in the sugarcane juices are susceptible to deterioration [3][5][6]. The deterioration of colour of sugarcane juice is mainly caused by polyphenol oxidase [7]. This enzyme changes the colour of juice and reduces the overall acceptability of the consumer. Moreover, the conversion of sucrose into polysaccharide such as dextran also contributes to the problem in ensuring good manufacturing process of sugarcane juice. The spoilage by microorganism mainly Leuconostoc spp. secrete endogenous invertase enzyme and activate this deterioration reaction in sugarcane juice [8]. According to Sigh et al., (2014), spoilage bacteria such as Leuconostoc spp. may enter the sugarcane by cuts and damaged site [6]. Hence, the synergistic effects of reactions during the postharvest period of sugarcane plant contribute fairly to the quality of the sugarcane juice.

In this study, the sugarcane juice is pasteurised and added with Spirulina platensis (S.platensis) water extract. After that, the effect of antioxidant characteristic of S.platensis water extract to the physicochemical and sensory properties of sugarcane juice for end user was further tested. The fact that S.platensis have antioxidant properties is supported by Miranda et al., (1998) as cited in Colla et al., (2007) that there are several phenolic compound that can be found in Spirulina which is salicylic, transcinnamic, synaptic, chlorogenic, quimic and caffeic acid are responsible for the antioxidant activity of the Spirulina species [9]. Hypothetically, with the supplementation of S. platensis water extract in sugarcane juice, the physicochemical and sensory properties as well as stability can be enhanced during the testing period.

2. Material and method
The sugarcane used in this study was collected from local vendor in Pagoh, Muar. The S. platensis commercial powder was used in this experiment (Merck, Germany). Peptone powder, plate count agar and potato dextrose agar were obtained from Merck (Germany).

2.1 Sample preparation
The fresh sugarcane samples were weighted, manually cleaned, washed, and cut into pieces with the same length. In this experiment, the samples were squeezed using mechanical sugarcane juice extractor with little amendment made from the study carried out by Jittanit et al. [10] The entire extracted samples were filtered using two layer of muslin cloth to remove the debris fragments. Then, the juices would undergo pasteurisation process at 70°C for 10 minutes in order to reduce the microbial load of the pre-treated juices. The S. platensis water extract of (0.5g) was added to the 250 mL of pasteurised sugarcane juice and aseptically filled into glass bottle. The S. platensis water extract was prepared according to the study by Abu Zaid et al. [11] with some modification. The Spirulina powder (1.5 g) was soaked in 100 mL ultrapure water and shaken (100 rpm) continuously for 24 hours at room temperature. The mixture then centrifuged at 4000 rpm for 20 minutes (at 4°C) and the supernatant was filtered (Whatman No. 1) to remove the cell debris. The filtrate was use as water extract. The water extract was exposed to UV light for 15 minutes to reduce risk of contamination and number of present microbes in the water extract since the extraction treatment did not imply heat treatment. The treated sugarcane juice was stored in refrigerator (4°C) and thawed at room temperature prior testing.

2.2. Determination of physicochemical properties of treated sugarcane juice
For the determination of pH value of treated sugarcane juice, a digital pH meter (Ph700, Eutech Instruments, UK) was used for the test [3]. Before analysis, the pH meter was calibrated using buffers of pH 4.0, 7.0 and 10.0 respectively. In the other hand, total soluble solid determination of treated sugarcane juice was determined using hand digital refractometer (PAL-BX/RI, Atago, Japan)[3].
drops of sugarcane juice was placed on the refractometer screen at 25°C, pressed and the value of soluble solid was expressed as Brix. Meanwhile, the determination of colour was carried out using UV VIS spectrophotometer (T60 UV VIS spectrophotometer) to assess the colour stability of the treated sugarcane juice scanned at 420 nm wavelength with slight amendmend from the study by Laksameethanasan et al. (2012) [12]. The turbidity measurement of sugarcane juice was carried out using UV VIS spectrophotometer (T60 UV VIS spectrophotometer) in order to assess the amount of suspended particles that may confer the juice’s deterioration condition. The procedure was carried out according to the study by Laksameethanasan et al., (2012) with a slight amendment, scanned at 900 nm wavelength [12].

2.3. Microbiological evaluation of treated sugarcane juice
The determination of microbial count using spread plate technique was used for enumeration of total plate count of sugarcane juice samples [3]. In total plate count, the serial dilution was carried out in universal bottle by mixing 1 mL of sample in 9 mL of peptone water using the universal bottle for 10⁻¹ dilution and continued until 10⁻⁶ dilution was obtained. 0.1 mL of mixed sample from 10⁻⁶ dilution was pipetted onto the centre of agar filled plate. The samples were spread using sterile glass spreader (hockey stick) over the surface of agar. The petri dish was invertedly incubated at 37°C for 48 hours in incubator and expressed as colony forming unit (CFU/mL) using equation 1, where, N is number of colony forming unit per ml; ΣC is total number of colony on plate; d is dilution which the first counts were obtained.

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N_{\text{CFU/mL}} = \frac{\Sigma C}{(1 \times 10^d)}
\]  

(1)

2.4. Sensory evaluation of treated sugarcane juice
For sensory evaluation of sugarcane juice, both control and treated samples, the standardised temperature of samples were set at 4°C for 4 hours before serving in paper cups. The sensory properties of sugarcane juice in one sitting were assessed by 30 semi-trained panellists (food technology students) of 30 people will used to assess the sensory properties of sugarcane juice in one sitting. The panellist need to rate the sample’s colour, aroma, sweetness, aftertaste, flavour and overall acceptability using Hedonic scale of 1 to 5 (1 = dislike extremely, 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, 5 = like extremely).

3. Results and discussion

3.1. Physicochemical properties of treated sugarcane juice
The effect of addition of S. platensis water extract on pH, total soluble solid (TSS), colour and turbidity of pasteurised control and pasteurised treated sugarcane juice were recorded within 5 weeks of storage are shown in Table 1. There is some significant difference in term of pH value of treated and control samples might be due to the loss of water soluble phycocyanin stability at pH 4.63 when stored at 4°C. According to Safari et al. [13] the stability of phycocyanin pigment is very closely affected by the storage time at various temperatures. The phycocyanin pigment has highest stability at pH 4.5 and temperature ranging from -18°C to 4°C and recommended to applied for food product that are stored in cold or freezing temperatures [13]. Since, the phycocyanin blue pigment is classified as open chain tetrapyrole pigments [14] there are possibility that this pigment may exist in different enantiomeric helical conformation [15] that affects the physicochemical properties of this tetrapyrrrole. Similar observation also reported by Eggleston [16] where the pH trend of sugarcane juice decreased in 14 days of storage which might be due to enzymatic action or spoilage microbes [16]. The reduction of microbial colonies’ number as the pH increased must be due to the effect of change of the cytoplasmic or intracellular pH toward their enzymatic activities, reaction rates, protein stability, structure of nucleic acid and biological molecules [17].

During the increasing pH of sugarcane juices in from week 2 to week 4, the colony occurrence in total plate count becomes reduced. This change could be a result of that during the storage of sugarcane
juice, the acidophiles spoilage microorganisms take control of the regulation of pH of sugarcane juice in week 2. From week 2 to week 4 the pH become increases and acidophiles microorganisms are greatly reduced and this phenomenon were caused by ‘base shock’ or called partial loss of pH homeostasis. Acidophiles are generally survives in highly acidic medium of range pH 0.5 to pH 5 in which their cytoplasmic membrane impermeable to proton in high extracellular pH environment. In addition, outside the pH permitting growth, the cell may die or inactive due to gradual failing of pH homeostasis. Thus, the number of viabilities may be reduce as shown in Table 2. Nevertheless, these microorganisms may revive again when their optimum pH of growth is reached again.

No significant different \((p>0.05)\) was reported on TSS, colour and turbidity of sugarcane juice when added with 0.2\% (vol/vol) of \(S. \ platensis\) water extract was added. Generally, the measurement for TSS in sugarcane juice ranging between 14° to 22° Brix, which showed the corresponding value of number of solids dissolved in sugarcane juice [3]. The increase in TSS pattern may due to the increased of sugar reduction activity in the juice via hydrolysis of total sugar [18] and utilisation of sugar component by microbes into organic acid. For colour assessment, colour absorbance at wavelength of 420 nm for sugarcane juice was chosen by referring to International Unit specified by International Commission for Uniform Methods of Sugar Analysis (ICUMSA) [19]. According to a previous study by Echavaria [20], the development of Maillard reaction can be assessed by analysing change of absorbance at 420 to 450 nm which measures the amount of final Maillard reaction product (MRPs) [20] and detects the activity of polyphenol oxidase and peroxidase enzymes that present readily in the sugarcane sample [21]. The coloured dissolved organic matter may result from Maillard reaction product (MRPs) which increased after the hydrolysis of sugar due to higher reaction rate between amino acid and simple sugar in Maillard reaction [22].

| Table 1: Effect of addition of \(S. \ platensis\) water extract in term of pH, total soluble solid (TSS), colour and turbidity in control and treated sugarcane juice. |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Weeks       | 0           | 1           | 2           | 3           | 4           | 5           |
| pH          | Control     | 5.34±0.04   | 5.18±0.02   | 4.73±0.07   | 4.85±0.05   | 5.26±0.02   | 5.13±0.04   |
|             | Treated     | 5.32±0.00   | 5.08±0.02   | 4.63±0.03   | 4.76±0.01   | 5.16±0.01   | 5.08±0.00   |
| TSS \(^{(*)}\)Brix | Control     | 14.5±0.06   | 14.4±0.00   | 13.9±0.17   | 13.6±0.21   | 14.5±0.12   | 14.5±0.29   |
|             | Treated     | 14.1±0.06   | 14.6±0.00   | 13.2±0.85   | 13.8±0.30   | 14.4±0.06   | 14.3±1.00   |
| Colour \((\text{Abs})\) | Control     | 1.557±0.01  | 2.172±0.00  | 0.797±0.06  | 1.482±0.00  | 1.757±0.01  | 2.183±0.02  |
|             | Treated     | 1.705±0.01  | 1.973±0.00  | 0.731±0.04  | 1.395±0.01  | 1.736±0.04  | 2.311±0.02  |
| Turbidity \((\text{Abs})\) | Control     | 0.375±0.00  | 0.728±0.00  | 0.310±0.03  | 0.395±0.01  | 0.502±0.07  | 0.774±0.01  |
|             | Treated     | 0.462±0.00  | 0.637±0.00  | 0.290±0.01  | 0.365±0.00  | 0.522±0.06  | 0.873±0.01  |

3.2. Microbial properties of treated sugarcane juice
The results for total plate count evaluation of control and treated sugarcane juice were recorded for 5 consecutive weeks were tabulated in Table 2. In the middle of storage period (week 3), high amount of colony forming unit (CFU/ml) was found in both tested samples. This could be resulting from the interaction between sugarcane juice medium and the ability of the microorganism surviving in high acidic medium as evidenced in a study by Lima, Grisi and Bonato [23]. The addition of \(S. \ platensis\) water extract in treated sample does not showing any significant different \((p>0.05)\) compared to the control sample. The results for the colony forming unit (CFU) is higher than control sample indicates that the water extract have little effect on the antimicrobial activity of sugarcane juice. The increasing of CFU count may result from the growth of acid tolerant microorganism which thrives in acidic environment and adapting to the available abundant nutrient in high acid medium such as simple sugars and organic acids such as glucose where most of the isolated bacterial were survive in glucose as sole carbon sources [23]. However, acidophiles is very sensitive to small molecular weight aliphatic acids such as acetic acid [26] where the reduction of growth of colony that might due to the accumulation of by-product in later stage of week.
Table 2: Total plate count for control sample and treated sugarcane juice samples.

| Weeks | TPC (CFU/ml) |
|-------|--------------|
|       | Control      | Treated      |
| 0     | 0            | 0            |
| 1     | 0            | 0            |
| 2     | 0            | 0            |
| 3     | 1.26 × 10^{10} | 2.96 × 10^{10} |
| 4     | 8.65 × 10^{9}  | 2.65 × 10^{9}  |
| 5     | 3.0 × 10^{7}   | 7.0 × 10^{7}   |

3.3. Sensory properties of treated sugarcane juice

From Table 3 and Figure 1, all sensory attributes of treated sample have higher score than control samples. However, there are no significant difference (p>0.05) when the sugarcane juice is added with *S. platensis* water extract except for colour attributes. Thus, it can be concluded that the control and treated sample do not have distinct difference except for colour attributes, in agreement with a study by Agustini *et al.* (2017), addition of *S. platensis* in enriched yogurt showed no significant different in term of appearance, flavour, taste, consistency and overall impression as compared to yogurt without *S. platensis* addition, even though the yogurt with *S. platensis* received best scores among tested samples [24]. Furthermore, the addition of 1% (w/w) of *S. platensis* biomass in croissant in a study by Massoud *et al.* [25] get the best score for sensory testing as compared to 0.5% (w/w) and 1.5% (w/w) of *S. platensis* biomass. Nevertheless, there are no significant different in term of attributes in three types of croissant (0.5% w/w, 1% w/w and 1.5% w/w of *S. platensis* biomass addition). From the previous study, it can be summarized that the introduction of *S. platensis* water extract does not change the attribute of the sugarcane juice and preference of the panellist.

Table 3: Sensory analysis for control and treated sugarcane juice samples.

| Parameter     | Mean Control     | Mean Treated    |
|---------------|------------------|-----------------|
| Colour        | 3.4667±0.78      | 4.000±0.87      |
| Aroma         | 3.3333±1.03      | 3.5667±0.90     |
| Sweetness     | 3.7000±1.00      | 3.7667±0.97     |
| Aftertaste    | 3.6667±1.06      | 3.8667±1.01     |
| Flavour       | 3.6333±1.00      | 3.8667±0.94     |
| Overall acceptability | 3.7333±0.87 | 3.9333±0.87 |

Figure 1: Comparison of sensory properties between control and treated sugarcane juice.

4. Conclusion

The physicochemical properties of sugarcane juice treated with *S. platensis* water extract have been successfully conducted in this research. It was found that, the analysis of pH, total soluble solid, colour and turbidity of sugarcane juice (both control and treated samples) have direct correlation with each
other except for microbiological assessment where the microbes appeared in the middle of testing period. The addition of *S. platensis* also have significant effect toward lowering the pH value of sugarcane juice in overall storage period for treated sample as compared to control samples. In the other hand, the sensory properties of sugarcane juice supplemented with *S. platensis* water extract also showed no significant difference in term of colour, aroma, flavour, sweetness, aftertaste and overall acceptability when compared to control sample. Nevertheless, the additions of *S. platensis* water extract into sugarcane juice scored better as compared to control sample.

From the study, *S. platensis* water extract have the potential as colouring agent in sugarcane juice since the addition of *S. platensis* does not affect the physicochemical properties and sensory acceptability of the sugarcane juice. The limitation on the assessment of sugarcane juice treated with *S. platensis* water extract is due insufficient time of assessment period to represent the whole view of changes in sugarcane juice samples in ambient storage condition. The physicochemical properties analysis of sugarcane juice by comparing to different demographic and variety can widen the perspective and information about changes that occur in sugarcane juice variety that available in Malaysia.

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